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BEARING ON HEREDITY AND VARIATION

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RECESSIVE RUMPLESSNESS OF FOWL WITH KYPHO-SCOLIOSIS AND SUPERNUMERARY RIBS

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Received January 27, 1945

DURING 1937 some abnormal chicks appeared among the progenies of two different breeding pens of our experimental flock of fowls. These chicks showed a close outward resemblance to specimens of the dominant rumpless variation and its intermediate modifications (DUNN 1925; LANDAUER and DUNN 1925; LANDAUER 1928). The simultaneous occurrence of a number of such rumpless birds suggested that we were dealing with a hereditary condition. There was no relationship to our dominant rumpless stock. The parents of these new rumpless chicks all had entirely normal skeletons. It appeared, therefore, that we were dealing with a recessive mutation.

Experiments were undertaken in order to learn something about the mode of inheritance and the morphological expression of the new mutation, and the results are to be reported here. A brief preliminary notice of this work was published in 1942.

MORPHOLOGY

The principal external features consist in an incomplete development or complete absence of the tail. The tail feathers may be reduced in number, or they may be entirely lacking. If tail feathers are present, they are generally in a drooping position and such birds are unable to carry their tails normally (fig. 1). The rump—that is, the fleshy part of the tail including free tail vertebrae and pygostyle—varies from complete absence to conditions which are, by palpation, indistinguishable from normal. The oil gland (uropygial gland) is generally lacking, but in those cases which most nearly approach normal development of the rump, the gland may be present as a rudiment or it may even be in a functional condition. With reference to all these traits, our material may be arranged in series which reach, by imperceptible steps, from complete absence of the structure in question to its normal differentiation.

The same intergradations from complete lack to normal morphological expression obtain for the caudal vertebrae and the pygostyle (fig. 2–9). In a certain proportion of genetically rumpless birds all tail vertebrae, including the pygostyle, are entirely normal. In other birds the only abnormality consists in a reduction in size of the pygostyle (fig. 5). Other animals have small caudal vertebrae with transverse processes which are reduced in size. In yet other individuals one or more of the five free caudal vertebrae may be absent, and some or all of these vertebrae may be rudimentary and fused together into an irregular bony knob which also includes the pygostyle (fig. 3, 4). In completely rumpless birds (fig. 2) one or two of the synsacro-caudal vertebrae, in addition to the caudal ones, are frequently lacking.

In all features which have just been enumerated, these new rumpless fowl resemble closely the rumpless and intermediate rumpless animals of the domi-

nant rumpless stock. There are, however, certain features which are distinctive of the new and, as will be shown subsequently, recessive mutation. One of these traits, very common among recessive rumpless animals but rare among the dominant ones, is that the fused caudal vertebrae are bent downward (caudal lordosis (fig. 3, 7)); another feature which is common among the recessive rumpless animals is that in cases with fusion of the caudal vertebrae the fused bony mass frequently appears compressed laterally—that is, the transverse diameter is much reduced (fig. 6); a third peculiarity is that in a certain number of recessive rumpless birds the anterior and posterior margins of the synsacro-caudal, and in some instances also of thoracic vertebrae, appear flaring, especially in ventral aspect. Neither one of the last two features has been observed in dominant rumplessness.

TABLE 1

Apparent number of synsacro-caudal and free caudal vertebrae, exclusive of pygostyle. Classification according to lateral-ventral processes.

NUMBER OF VERTEBRAE	335 WHITE LEGHORN ♂♂ %	133 R.I. RED ♂♂ %	160 R.I. RED ♀♀ %
11	0	0	0.6
10	44.5	39.1	48.8
9	54.6	60.2	50.6
8	0.9	0.8	0

The fusion of caudal vertebrae frequently precludes an exact vertebral count. Moreover, a precise determination of the number of caudal vertebrae is often attended by difficulties even in the absence of fusion on account of considerable variation at the boundary of the synsacro-sacral and the synsacro-caudal regions. According to DU TOIT's (1913) classification, which is here followed, the synsacrum of Gallus is normally composed of the following vertebrae: one synsacro-thoracic, four synsacro-thoraco-lumbar, four synsacro-lumbar, two synsacro-sacral, and five synsacro-caudal.

In addition, there are five free caudal vertebrae and the pygostyle. It happens frequently, however, that the first synsacro-caudal vertebra bears lateral-ventral processes, thereby assuming the appearance of a synsacro-sacral vertebra. At the same time, the last synsacro-sacral vertebra may have lost its lateral-ventral processes, thus resembling a synsacro-lumbar vertebra. In the latter cases, we are dealing with "sacralization" of a synsacro-caudal vertebra, and the number of *typical* synsacro-caudal vertebrae is thereby reduced to four. This accounts for the fact that in 50 to 60 per cent of all cases of normal White Leghorn and Rhode Island Red fowl which we dissected, only four synsacro-caudal vertebrae appeared to exist (table 1). It has been shown by PROMPTOFF (1928–1929) that there is a high degree of variability in the number of synsacro-thoracic and synsacro-thoraco-lumbar vertebrae of fowl and that these variations have a genetic basis We have not inquired into the relation, if any,

which may exist between the vertebral variants of the two synsacral regions.

Bearing in mind the difficulties of enumerating the exact number of synsacro-caudal and caudal vertebrae, an attempt has nevertheless been made to estimate in a semi-quantitative manner the degree of development and the presence or absence of the vertebrae in question (table 2). The tabulated material comprises all adult progeny from our 1942 and 1943 *inter se* matings of

TABLE 2

Percentage distribution of adult animals of the recessive rumpless stock according to development of the caudal vertebrae. The figures in parentheses represent the number of individuals with only three or four synsacro-caudal vertebrae.

	1942		1943	
	216 ♀♀	172 ♂♂	196 ♀♀	125 ♂♂
1. Vertebrae normal	31.5 (1)	26.7 (1)	29.6 (2)	24.0 (10)
2. Pygostyle abnormal	24.1	12.8 (2)	25.0 (9)	19.2 (15)
3. Fifth caudal vertebra missing or abnormal and/or fused with rudimentary pygostyle	13.0	7.6	9.2 (6)	7.2 (7)
4. Fourth and fifth caudal vertebrae fused with rudimentary pygostyle	4.2	0.6	6.1 (2)	8.8 (1)
5. First two caudal vertebrae normal, others rudimentary and/or fused with rudimentary pygostyle	2.3 (1)	3.5	5.1 (3)	8.0 (9)
6. First caudal vertebra normal, others rudimentary and/or fused with rudimentary pygostyle	5.1	1.7 (1)	3.6 (1)	6.4 (3)
7. All caudal vertebrae and pygostyle rudimentary (some may be missing)	17.6 (11)	44.8 (33)	17.3 (19)	22.4 (20)
8. All caudal vertebrae, including pygostyle, missing	0	0	1.0	0
9. All caudal and one or more synsacro-caudal vertebrae missing	2.3	2.3	3.1	4.0

recessive rumpless birds. The groups (1–9) are arranged according to an increasing degree of abnormality, beginning with animals which have entirely normal tail vertebrae (some of these, however, had other abnormalities, as will be discussed below) and ending with those in which all caudal and some synsacro-caudal vertebrae are lacking. The extent of reduction in size of particular vertebrae or their parts (for example, transverse processes) does not find expression in this summary, nor does the degree of fusion or of abnormality of formation.

It may be seen that an appreciable proportion of the animals show nothing but a reduction in size and shape of the pygostyle. In many instances of this kind the posterior tip of the pygostyle is absent, its end being blunt instead of pointed. Among the cases of more extreme reduction of the tail those are most common in which all caudal vertebrae are reduced in size and fused together into a bony knob. Complete absence of all caudal vertebrae is relatively uncommon in this material.

The most striking feature of these data is a difference in distribution be-

tween the sexes. This may be seen more clearly if the groups are summarized into larger categories (table 3) It is evident that a nearly normal differentiation is much more common among females than males or, conversely, that the males tend to show more extreme abnormality of the tail skeleton. The absence of one or two synsacro-caudal vertebrae (or a "sacralization" of one or two vertebrae of this region, producing the appearance of loss) is also more frequent among males than among females.

TABLE 3

Totals of sub-groups in table 2 in percentage.

SUB-GROUPS	1942		1943	
	♀	♂	♀	♂
1–3	68.6	47.1	63.8	50.4
4–7	29.2	50.6	32.1	45.6
8–9	2.3	2.3	4.1	4.0
Only 3 or 4 instead of 5 synsacro-caudal vertebrae	6.0	21.5	21.4	52.0

SCOLIOSIS, LORDOSIS, AND SUPERNUMERARY RIBS

Two abnormalities which occur rather frequently in the recessive rumpless stock are scoliosis and supernumerary ribs.

As far as scoliosis is concerned, we are dealing with cases of typical kypho-scoliosis. The extent of lateral deformation of the vertebral column varies from slight deviations, which can barely be recognized, to extreme bends at about right angles (fig. 8–11). The apex of the main curvature may be located as far anteriorly as the first synsacro-thoracic vertebra; it may be as far posteriorly as the fourth synsacro-thoraco-lumbar vertebra; but it occurs most commonly at the level of the second synsacro-lumbar. A compensatory scoliosis in the opposite direction is generally found in the region of the thoracic vertebrae. It is most frequently located at the level of the sixth thoracic vertebra, at the boundary of the sixth and seventh, at the level of the seventh, or between the latter and the synsacro-thoracic vertebra.

EXPLANATION OF FIGURES 1–9

FIGURE 1.—Recessive rumpless ♂3062 with intermediate rumplessness.

FIGURE 2.—Pelvic bones of ♂3245. Dorsal aspect. Completely rumpless.

FIGURE 3.—Dorsal view of pelvic bones of ♂1156. Small rudiment of fused caudal vertebrae. Slight lordosis of caudal vertebrae.

FIGURE 4.—Dorsal aspect of pelvic bones of ♂3238. Fused caudal vertebrae.

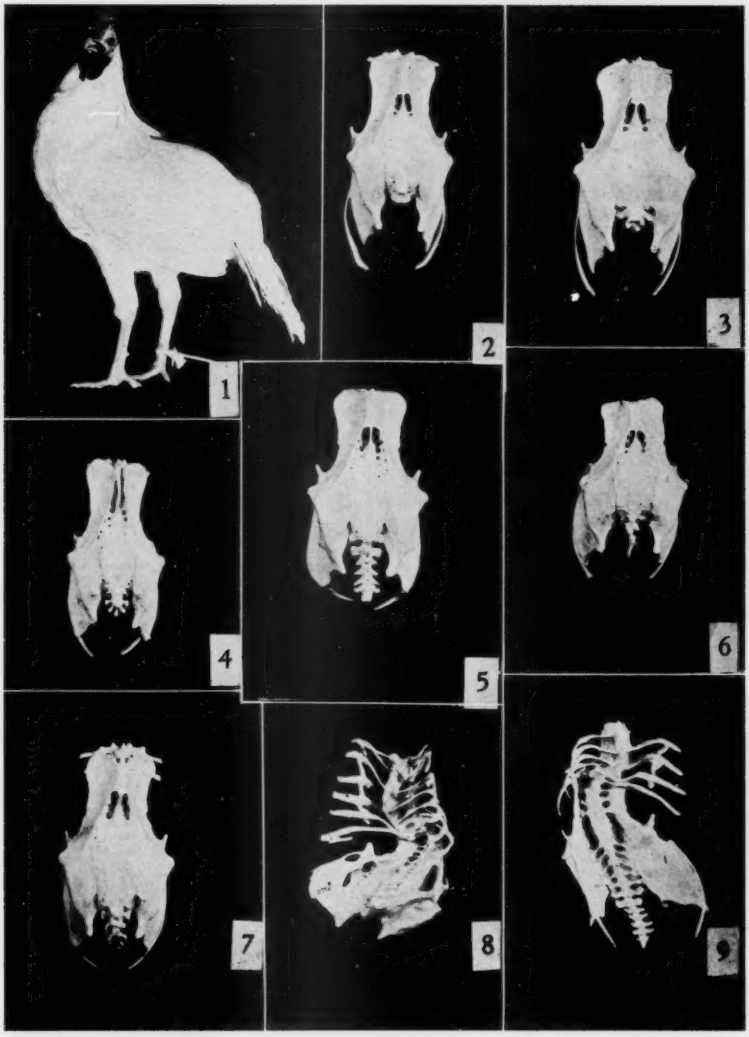
FIGURE 5.—Dorsal view of pelvic bones of ♂1546. Pygostyle rudimentary, other caudal vertebrae normal.

FIGURE 6.—Pelvic bones of ♀4775 from dorsal side. Caudal vertebrae abnormal, partially fused, compressed laterally. Lordosis of caudal vertebrae.

FIGURE 7.—Dorsal aspect of pelvic bones of ♂3247. Partial fusion and lordosis of caudal vertebrae.

FIGURE 8.—Ventral view of pelvic bones of ♂3252. Small rudiment of caudal vertebrae. Extreme kypho-scoliosis. Rudimentary rib on right body side.

FIGURE 9.—Ventral aspect of pelvic bones of ♀5720. Pygostyle rudimentary, other caudal vertebrae normal. Kypho-scoliosis.



FIGURES 1–9

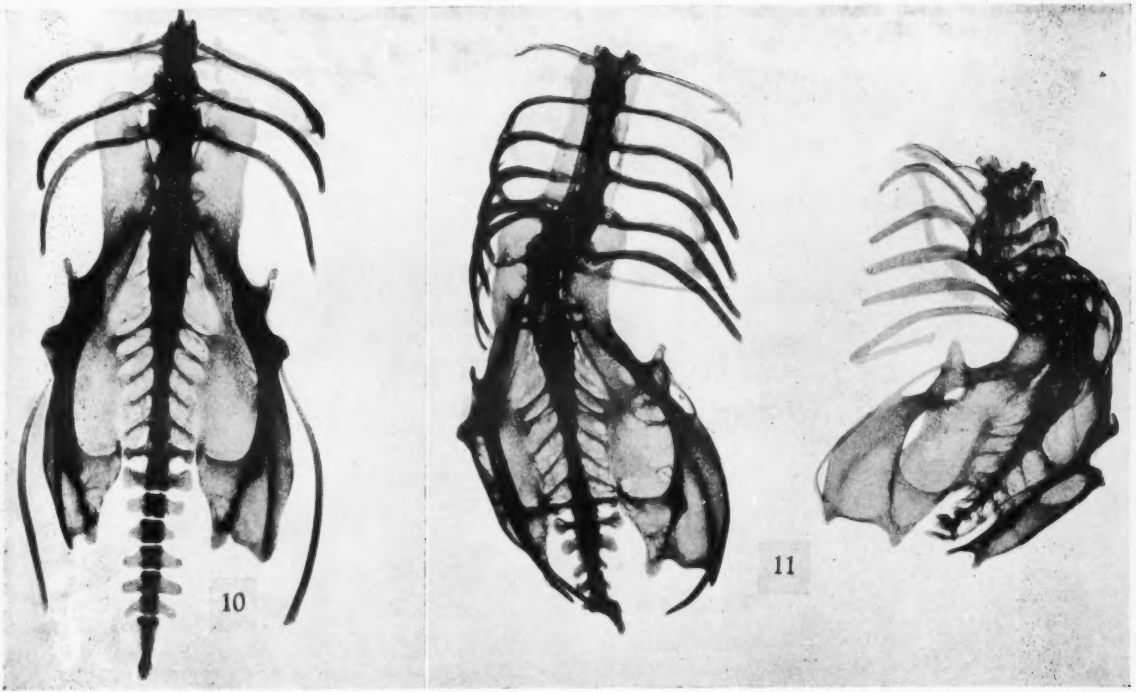


FIGURE 10.—X-ray picture of pelvic bones of a normal White Leghorn male.

FIGURE 11.—X-ray pictures of pelvic bones of ♂8967 (left) and ♂3252 (right). The caudal vertebrae of ♂8967 are better developed than those of ♂3252. There is marked kypho-scoliosis in both. ♂8967 has complete supernumerary ribs on both sides; ♂3252 has a rudimentary extra rib on the right body side.

There is generally a more or less pronounced kyphosis near the apex of the main curvature of the spine. Within the synsacrum the bodies of the vertebrae are fused; hence, no abnormalities can be seen in shape of the vertebral bodies. Their lateral processes, on the other hand, frequently are abnormal in shape and position. In the region of the compensatory thoracic scoliosis the bodies of the vertebrae, especially their joint surfaces are often deformed. In extreme cases one or two of the thoracic vertebrae may be wedge-shaped. There is considerable torsion of the spine, which is most pronounced at the height of the curvatures. HÄRTEL (1909) has given an excellent description of the morphological details in ten cases of kypho-scoliosis in fowl; our observations agree entirely with his, and further details may be found in his publication.

The convexity of the main curvature of the spine may be either toward the right or left side of the body axis. In each of five samples for which we have data, there was a preponderance of cases with curvatures toward the left side. Among a total of 28 females with scoliosis the convexity of the synsacral curvature was toward the left in 23 cases, and out of 25 males 15 had this type of asymmetry.

It is chiefly on account of the kyphosis that the pelvic bones participate in the abnormality. The acetabulum is always higher on the side of the scoliotic convexity than on the opposite side. In spite of this lateral slanting of the pelvis, the gait is quite normal even in those animals with the most extreme abnormality. The explanation for this situation is found in the presence of coxa vara of that femur which articulates with the lower one of the two acetabula. A comparison of the femora in such cases shows that on the side on which the acetabulum stands lower, the caput femoris is flattened on its upper surface and, instead of pointing upward, forms a nearly right angle with the proximal head of the femur. Measurements of femur length, taken on the external side (highest point of trochanter to most distal point of condylus externus), give similar results for the two femora. If, however, femur length is measured on the internal side (highest point of caput femoris to most distal point of condylus internus), significant differences between the two femora are found Among 21 scoliotic females the femur shortening due to coxa vara amounted to 1.2 ± 0.1 mm, and for 13 males the corresponding figure was 1.7 ± 0.2 mm. Corrective (functional) adaptation of locomotion to the pelvic abnormality is thus brought about by a typical "Belastungsdeformität"—namely, coxa vara.

Scoliosis in this material does not occur with equal frequency among the two sexes, as is shown by the data of table 4. In each of three successive years males had a much higher incidence of scoliosis than females. The significance of this sexual difference will be discussed later.

An appreciation of the frequency of scoliosis in the recessive rumpless material is afforded by the fact that in control observations one case of scoliosis was found among 335 White Leghorn fowl (0.3 per cent) and one among 293 Rhode Island Red chickens (0.3 per cent).

Next, it is of interest to inquire into the relationship between kypho-scoliosis and abnormalities of the caudal skeleton. Pertinent data from 838 chickens of our recessive rumpless stock are assembled in table 5. Some cases of scoliosis

were found in animals whose caudal skeletons were entirely normal. The frequency of scoliosis in this group is much higher than among genetically normal (that is, non-rumpless) chickens, but it is also much lower than in animals which have, in addition, some degree of abnormality of the caudal vertebrae. Again, among those chickens in which the free caudal vertebrae are abnormal or lacking there is a much higher incidence of scoliosis than among those with a mere reduction in size of the pygostyle. All these conclusions apply to males

TABLE 4

*Frequency of kypho-scoliosis in the recessive rumpless stock according to sex.**

YEAR	FEMALES		MALES	
	NUMBER OF ANIMALS OBSERVED	PERCENTAGE OF CASES WITH KYPHO-SCOLIOSIS	NUMBER OF ANIMALS OBSERVED	PERCENTAGE OF CASES WITH KYPHO-SCOLIOSIS
1941	154	35.7±3.9	126	54.8±4.4
1942	216	25.9±3.0	171	46.2±3.8
1943	196	23.5±3.0	126	35.7±4.2

* Standard errors are used throughout this report.

TABLE 5

Frequency of kypho-scoliosis according to the degree of abnormality of the caudal skeleton and according to sex.

CONDITION OF CAUDAL SKELETON	365 ♂♂		473 ♀♀	
	NUMBER OF ANIMALS	PERCENTAGE WITH KYPHO-SCOLIOSIS	NUMBER OF ANIMALS	PERCENTAGE WITH KYPHO-SCOLIOSIS
Normal	99	8.1±2.7	146	2.1±1.2
Free caudal vertebrae normal, but small pygostyle	59	28.8±5.9	109	23.9±4.1
Free caudal vertebrae and pygostyle abnormal or lacking	207	56.5±3.5	218	41.3±3.3
Total	365	38.9±2.5	473	25.2±2.0

as well as females. It appears, therefore, that there is a developmental correlation between the degree of abnormality of the caudal skeleton and the occurrence of kypho-scoliosis. The significance of this relationship will be discussed later.

In an appreciable percentage of those animals of the recessive rumpless stock which have rudimentary and fused caudal vertebrae the fused caudal mass is bent toward the ventral side. This lordosis was observed in 17.0±2.0 per cent of males (N=370) and in 12.1±1.5 percent of females (N=480). The difference in sexual incidence is not statistically significant, but is in the same direction as that found for other traits, indicating a trend toward more frequent abnormality among the males.

Another peculiarity of our recessive rumpless stock is the presence of supernumerary ribs in a certain percentage of the animals. Such supernumerary ribs occur in fowl of normal breeds. For instance, among 335 White Leghorn cockerels there were nine or 2.7 per cent, among 133 Rhode Island Red males there were ten or 7.5 per cent, and among 160 Rhode Island Red females there were eight or 5.0 per cent of animals with supernumerary ribs.

These extra ribs are posterior to the last pair of normal thoracic ribs (fig. 8, 11). They may occur bilaterally or on only one side. The vertebral part of the supernumerary ribs may be completely formed or rudimentary. No sign of sternal parts has ever been seen. If rudiments of ribs are present, it is always the distal parts which are found. These rib rudiments may be connected with the vertebral column by connective tissue condensations. Even if the supernumerary ribs are complete, they are very slender.

TABLE 6

Frequency of supernumerary ribs in the recessive rumpless stock according to degree of expression and according to sex.

DEGREE OF DEVELOPMENT			
ACCORDING TO BODY SIZE	COMPLETE OR RUDIMENTARY	369 ♂♂	473 ♀♀
Both sides	Complete	1.90	0.42
	Complete on one side, rudimentary on other	0.54	0.42
	Rudimentary	4.61	2.33
On left	Complete	1.63	0.21
	Rudimentary	4.07	1.69
On right	Complete	1.63	0.21
	Rudimentary	2.98	2.75
Total		17.34±1.97	8.03±1.25

A summary of the occurrence of supernumerary ribs among 842 animals of the recessive rumpless stock is presented in table 6. It can be seen that supernumerary ribs are significantly more frequent in males than in females, that the rudimentary ribs are found more often unilaterally than bilaterally, and that they are more commonly rudimentary than complete. For the total of all types of supernumerary ribs the frequency is much greater than in non-rumpless stock (White Leghorn and Rhode Island Red). Among cases with unilateral supernumerary ribs no definite preponderance of either left or right body side is apparent.

The data in table 7 give some information concerning the relation between supernumerary ribs and the degree of abnormality, if any, of the caudal skeleton. Among those animals which had an entirely normal tail skeleton the frequency of supernumerary ribs did not differ significantly from that found in control stock. On the other hand, among all animals with abnormal caudal vertebrae the incidence of supernumerary ribs was greatly increased, irre-

spective of whether there was only a reduction in size of the pygostyle or more extreme deformity. A separate consideration of animals with scoliosis shows that among them, without regard to the presence or absence of abnormalities of the caudal skeleton, supernumerary ribs occur still more frequently than in the groups discussed previously. This indicates, presumably, that the two conditions have certain developmental events in common.

TABLE 7

Frequency of supernumerary ribs, either rudimentary or complete, according to the degree of abnormality of the caudal skeleton and according to sex.

CONDITION OF CAUDAL SKELETON	365 ♂♂		473 ♀♀	
	NUMBER OF ANIMALS	PERCENTAGE WITH SUPERNUMERARY RIBS	NUMBER OF ANIMALS	PERCENTAGE WITH SUPERNUMERARY RIBS
Normal	99	9.1±2.9	146	1.4±1.0
Free caudal vertebrae normal, but small pygostyle	59	20.3±5.2	109	9.2±2.8
Free caudal vertebrae and pygostyle abnormal or lacking	207	21.3±2.9	218	11.9±2.2
Total	365	17.8±2.0	473	8.0±1.3
Scoliosis with or without abnormality of caudal skeleton	101	32.7±4.7	71	21.1±4.9

GENETICS

Several partially rumpless birds were found in 1937 in matings of Creeper and normal fowl which had descended from crosses of Creepers and White Leghorns. In the same year a few birds of similar appearance segregated in a mating of Hamburg×Leghorn crossbred fowl. Every bird in these different matings had in its ancestry White Leghorn stock which had been purchased in 1933 from a commercial hatchery. No other stock was common to the ancestry of all the rumpless birds. An appreciable amount of inbreeding had occurred in the history of every one of the rumpless animals, and the rumpless mutation found in the Creeper material was genetically identical with that recovered from the Hamburg cross. It may be concluded that the rumpless variation was derived from the White Leghorn stock. Further evidence in favor of this conclusion may be seen in the fact that some partially rumpless chickens which DR. D. C. WARREN obtained from a breeder of White Leghorn fowl in the state of Washington and which were placed at the disposal of the writer, on crossing with our material proved to be genetically identical as far as the rumpless mutation was concerned.

In 1938 we bred one of our new rumpless cockerels to normal White Leghorn hens. These matings resulted in a progeny of 102 animals, all normal. During the following three years we continued to breed this stock with the results shown in table 8. For reasons which will become clear from the subsequent discussion, the recessive rumpless mutation which is the subject of this

report will be designated as *rp2*, whereas the symbol *Rp1* will be used for the dominant rumpless mutation of our earlier publications.

The data in table 8 are based on gross observations of growing and adult chickens and of embryos which died during the last four days of incubation Later experience has shown that classification based on palpation is not entirely reliable, since the intermediate rumpless condition may so closely approach the normal as to be indistinguishable, except by dissection In fact, as

TABLE 8

Segregation in various rumpless matings. Classification by palpation.

PEN	YEAR	TYPE OF MATING	TOTAL OF CLASSIFIED PROGENY	NORMAL PERCENTAGE	RUMPLESS AND INTERMEDIATE RUMPLESS—PERCENTAGE
10	1939	F1 *inter se*	783	93.5	6.5
14	1939	F1 *inter se*	940	94.8	5.2
18	1940	F1 ♀ ♀ × *rp2*♂	349	81.5	18.5
18	1940	Normal F2 ♀ ♀ × *rp2*♂	158	83.5	16.5
15	1940	*rp2 inter se*	180	66.1	33.9
3	1941	*rp2 inter se*	45	66.7	33.3
18	1941	*rp2 inter se*	274	62.0	38.0

will be seen later, birds which are homozygous for the rumpless condition may yet have an entirely normal caudal skeleton. It follows that the figures given in table 8 are minimal values as far as rumpless frequency is concerned.

With these limitations of the data in mind, our observations show that *inter se* matings of F1 birds from crosses of Leghorn and intermediate rumpless fowl produced 5.2 per cent and 6.5 per cent of rumpless progeny, respectively, in the F2 generations. A backcross of F1 females to an intermediate rumpless male and a mating of "normal" F2 females (from the F1 *inter se* matings of the preceding year) to an intermediate rumpless male gave nearly identical percentages of rumpless progeny (18.5 and 16.5 per cent, respectively). The majority of the "normal" females were presumably heterozygous for the rumpless mutation, some were probably genetically normal, and a few may actually have been homozygous rumpless. Three families of intermediate rumpless birds produced between 33.3 and 38.0 per cent of rumpless and intermediate rumpless progeny.

All crosses of genetically normal and rumpless birds indicate complete recessiveness of the rumpless condition, but in backcrosses as well as in F2 and later generations there is always a great deficiency of rumpless and intermediate rumpless offspring. It appears, therefore, that the "penetrance," as well as the "expressivity," of our new rumpless mutation is low. There is every reason to believe that these peculiarities are due to multiple modifiers which may suppress the effect of the rumpless mutation either partially or completely.

In an effort to eliminate these modifiers, at least in part, we used for matings in subsequent years the offspring of parents with marked abnormality of the caudal skeleton. At the same time it was decided to base all classification on results of dissection. There is no doubt that a certain number of cases which

would have been classified as normal by palpation were, on dissection, found to be intermediate rumpless. There is also little doubt, however, that the marked increase in intermediate rumpless progeny (table 9, 10) was due chiefly to selection. This is shown by the data of table 9. Some of the breeding females

TABLE 9

Segregation in matings of progeny from intermediate rumpless parents. The males in all matings were intermediate rumpless. All females in pens 12 and 17 were intermediate rumpless. The females in pen 18 appeared normal from external examination, but on dissection a part of them were found to be intermediate rumpless. The classification of parents and progeny is based on dissection.

PEN	YEAR	PARENTS	PROGENY		
			TOTAL	NORMAL PERCENTAGE	RUMPLESS OR INTERMEDIATE RUMPLESS—PERCENTAGE
12	1942	Intermediate rumpless	50	20.0	80.0
17	1942	Intermediate rumpless	70	21.4	78.6
18	1942	Females intermediate rumpless, but abnormality of caudal skeleton confined to pygostyle	77	44.2	55.8
		Females normal	70	40.0	60.0

TABLE 10

Results of matings in 1943. The males used were intermediate rumpless. The females consisted of groups of normal and intermediate rumpless sisters from intermediate rumpless parents. Classification of parents and progeny according to dissection.

MOTHERS		PROGENY		
NUMBER	CAUDAL SKELETON	TOTAL	NORMAL PERCENTAGE	RUMPLESS AND INTERMEDIATE RUMPLESS PERCENTAGE
10	Normal	88	34.1	65.9±5.0
7	Pygostyle small, otherwise normal	92	13.0	87.0±3.5
10	Intermediate rumpless*	85	21.2	78.8±4.6

* Including one rumpless female with two intermediate rumpless progeny.

in pen 18 had an entirely normal caudal skeleton; others were intermediate rumpless, but the abnormality was so slight that it could not be discovered by palpation. These two groups of females produced virtually the same percentage of rumpless and intermediate rumpless progeny (60.0 and 55.8 per cent, respectively). The incidence of rumplessness among their progeny was definitely lower than among the offspring of parents with marked abnormalities of the caudal vertebrae (pens 12 and 17).

In 1943 we chose for our matings groups of sisters which either had a normal caudal skeleton or showed varying degrees of abnormality of the caudal vertebrae. They were bred to the same cocks. The progenies of the two groups of

intermediate rumpless mothers (table 10) did not differ significantly in incidence of rumpless and intermediate rumpless animals; together they had 83.1 ± 2.8 per cent of offspring with abnormalities of the caudal skeleton. The normal mothers, on the other hand, had but 65.9 ± 5.0 per cent of rumpless and intermediate rumpless progeny. The difference between the two groups amounts to 17.2 ± 5.7 per cent and is significant.

The following conclusions can be drawn from our breeding experiments. The new rumpless mutation is completely recessive in all stocks with which it has been crossed. The expression of the mutation varies from entire absence of all caudal vertebrae to conditions which are, even by dissection, indistinguishable from an entirely normal caudal skeleton. The incidence of abnormal progeny ("penetrance") and the individual degree of abnormality ("expressivity") can be increased by selection, indicating the existence of modifying genes which counteract cumulatively the effects of the mutation itself. The final proof that the phenotypically normal animals are genotypically rumpless has been provided by the developmental studies of ZWILLING (1945) in which it was demonstrated that *all* embryos from matings of intermediate rumpless fowl do in certain stages show abnormalities of differentiation of the tail region, and that some of these embryos subsequently become normal.

Our crosses of rumpless males to Leghorn females produced only normal progeny. It must be concluded that the rumpless gene is located in one of the autosomes. There is some evidence, however, which suggests that some of the modifiers of rumplessness may be sex-linked. This will be discussed below.

It was demonstrated above that kypho-scoliosis (table 5) and supernumerary ribs (table 6) occur but rarely in those genetically rumpless animals which have normal caudal vertebrae. In fact, both of these abnormalities are found most frequently among fowl in which the caudal vertebrae are either completely lacking or in a very rudimentary condition. As we progress to less abnormal conditions of the caudal skeleton, the incidence of kypho-scoliosis and of supernumerary ribs decreases, although it must be emphasized that kypho-scoliosis as well as supernumerary ribs are in rare instances found in animals with entirely normal caudal vertebrae. This situation suggests that all of these abnormalities have causal physiological agencies in common.

Some further insight may be gained from the results of matings presented in tables 11 and 12. These data show that the frequency of kypho-scoliosis does not differ significantly between matings in which only one parent had kypho-scoliosis and others in which both parents were affected with it. Similarly, there is no significant difference in incidence of supernumerary ribs between matings in which neither parent, one or both of them had supernumerary ribs. These results militate against the conclusion that either kypho-scoliosis or supernumerary ribs are determined by genetic factors which are independent of rumplessness. Such results, on the other hand, fit well into the concept that kypho-scoliosis and supernumerary ribs are parts of a syndrome the principal feature of which is rumplessness. This conclusion is also strengthened by the fact that the matings which have just been discussed did not produce significant differences in the incidence of rumpless or intermediate rumpless progeny.

TABLE 11

Segregation data of various matings involving kypho-scoliosis.

PARENTS		PROGENY		
MOTHER	FATHER	TOTAL	SCOLIOSIS PERCENTAGE	RUMPLESS AND INTERMEDIATE RUMPLESS PERCENTAGE
Scoliosis	No Scoliosis	15	53.3	80.0
No scoliosis	Scoliosis	207	31.9	64.7
Scoliosis	Scoliosis	103	29.1	78.6

TABLE 12

Segregation data of various matings involving the occurrence of supernumerary ribs.

PARENTS		PROGENY		
MOTHER	FATHER	TOTAL	EXTRA RIBS PERCENTAGE	RUMPLESS AND INTERMEDIATE RUMPLESS PERCENTAGE
No extra ribs	No extra ribs	228	14.5	75.0
Extra ribs	No extra ribs	37	24.3	70.3
No extra ribs	Extra ribs	271	9.6	65.3
Extra ribs	Extra ribs	23	8.7	56.5

VIABILITY

The results of incubation for the matings involving our new rumpless mutation are brought together in table 13. It can be seen that in many of the *inter se* matings of rumpless fowl embryonic mortality is somewhat higher than it was in the original cross of Leghorn×rumpless or in the two mating of F1 birds. This increased mortality is due chiefly, if not entirely, to inbreeding, since a cross of rumpless females from our stock to an unrelated rumpless male, obtained from DR. D. C. WARREN, brought the hatching results back to the level of the F1 families (pen 16—1943). The females in this mating were sisters and half-sisters of those in pens 17—1943 and 18—1943.

It is true, however, that the percentage of completely rumpless animals is higher among the embryos which failed to hatch than among hatched chicks. This suggests that, similar to what is true for the dominant rumpless mutation (DUNN and LANDAUER 1934), embryonic mortality of the extreme rumpless variants is slightly higher than normal.

A more pronounced selective mortality occurs after hatching. The data for three years on mortality among progenies of rumpless matings during the first two months after hatching are shown in table 14. These results are tabulated according to the phenotypic appearance of the chicks at hatching time. Classification was done by palpation and is reasonably accurate as judged by later

TABLE 13

Embryonic mortality and hatching results in matings involving the recessive rumpless mutation.

MATING	NUMBER OF FERTILE EGGS	MORTALITY DURING INCUBATION DAYS, PERCENTAGE			HATCHED PERCENTAGE
		1–6	7–17	18–22	
Leghorn ♀♀ × *rp2*♂ (10—1938)	109	2.8	3.7	4.6	89.0
F1 *inter se* (14—1939)	1068	3.4	2.4	6.8	87.5
F1 *inter se* (10—1939)	930	3.6	3.2	8.1	85.2
F1 ♀♀ × *rp2*♂ (18—1940)	547	6.4	3.3	9.9	80.4
rp2 × *rp2* (15—1940)	212	3.8	9.4	7.6	79.2
rp2 × *rp2* (3—1941)	335	5.4	7.2	13.7	73.7
rp2 × *rp2* (12—1942)	78	5.1	6.4	7.7	80.8
rp2 × *rp2* (17—1942)	264	11.4	8.7	11.7	68.2
rp2 ♀♀ with normal caudal skeleton, × *rp2*♂ (18—1942)	115	6.1	6.1	6.1	81.7
rp2 ♀♀ with abnormal pygostyle × *rp2*♂ (18—1942)	152	5.9	4.6	3.9	85.5
rp2 × *rp2* (16—1943)	108	2.8	0	12.0	85.2
rp2 × *rp2* (17—1943)	394	5.3	6.8	13.7	74.1
rp2 × *rp2* (18—1943)	239	9.6	8.0	10.5	72.0

TABLE 14

Mortality during the first two months after hatching of recessive rumpless chicks which had either a phenotypically normal caudal skeleton or were rumpless and intermediate rumpless. Classification by palpation at hatching time.

YEAR	NORMAL CAUDAL VERTEBRAE		RUMPLESS AND INTERMEDIATE RUMPLESS	
	HATCHED	MORTALITY FIRST TWO MONTHS PERCENTAGE	HATCHED	MORTALITY FIRST TWO MONTHS PERCENTAGE
1942	133	19.5	393	21.1
1943	73	12.3	316	20.3
1944	292	11.6	376	20.2
Total	498	13.9±1.5	1085	20.6±1.2

dissection. It may be seen that the mortality of rumpless and intermediate rumpless chicks is greater than that of their normal (genetically rumpless) sibs, the difference amounting to 6.7 ± 1.9 per cent. The mortality among the phenotypically normal chicks is similar to that among other stocks raised under the same conditions.

The heavier mortality of rumpless and intermediate rumpless chicks after hatching appears to affect males more than females. This is suggested by the fact that there was an excess of females in every adult group of rumpless fowl which has been discussed in this report More direct evidence on this point was obtained during 1944 when all chicks which died between hatching and maturity were sexed. It was found that among 47 phenotypically normal chicks from the rumpless stock 22 were males and 25 females—that is, virtual equality; on the other hand, among 96 rumpless and intermediate rumpless chicks which had come from the same matings and had died during the same period, 65 were males and only 31 were females. This greater mortality of rumpless and intermediate rumpless males is not surprising, since it was found that males are definitely more affected by this mutation than females.

CALCIFICATION OF THE SKELETON

Some of the dissected pelvic bones of recessive rumpless fowl appeared to be unusually thin and poorly calcified. It was decided, therefore, to determine the relative amounts of bone ash and bone calcium in the pelvic bones of such animals. For comparison with the pelvis, similar determinations were made for one femur and one tibia of each bird. For comparison with the recessive rumpless material, the same bones were analyzed from rumpless (or intermediate rumpless) and normal birds of the dominant rumpless stock. All bones came from mature cockerels.

The results of these analyses are given in tables 15 and 16. In evaluating these data it should be noted that the normal birds from the dominant rumpless stock were genetically normal (that is, non-rumpless), whereas the cockerels with normal caudal vertebrae, which came from our recessive rumpless matings, were genetically rumpless. All families were derived from outcrosses to the same White Leghorn stock. Body weight, and consequently bone weight, was much greater in the dominant than in the recessive rumpless families. This indicates, presumably, that the two stock were not isogenic.

The data of table 16 demonstrate that the pelvic bones of animals from the recessive rumpless stock have a significantly lower ash and calcium content, in percentage of fresh weight, than do the pelvic bones of cockerels from the dominant rumpless strain. The differences between the means are as follows:

Bone ash in pelvis in percentage of fresh weight

Dominant rumpless less recessive rumpless	$2.17 \pm 0.63\%$
Normal from dominant rumpless less recessive rumpless with normal caudal skeleton	$2.35 \pm 0.56\%$

Bone calcium in pelvis in percentage of fresh weight

Dominant rumpless less recessive rumpless	$0.92 \pm 0.24\%$
Normal from dominant rumpless less recessive rumpless with normal caudal skeleton	$0.93 \pm 0.21\%$

The means for percentage ash in the dry, extracted bone and for percentage calcium in the ash of the pelvic bones show analogous, if less great, differences. There is, on the other hand, no comparable dissimilarity with regard to the femur or tibia.

It appears, therefore, that the pelvic bones of birds from the recessive rumpless stock are deficient in bone ash and bone calcium, and this is true irrespective of the presence or absence of abnormalities of the caudal skeleton.

TABLE 15

Fresh weight and dry weight in percentage of fresh weight of femur, tibia, and pelvis of mature cockerels from the dominant and recessive rumpless stock.

GROUP	NUMBER	FRESH WEIGHT IN G.			DRY WEIGHT IN PERCENTAGE OF FRESH WEIGHT		
		FEMUR	TIBIA	PELVIS	FEMUR	TIBIA	PELVIS
Dominant rumpless (complete or intermediate)	15	15.4±0.6	18.9±0.7	31.1±1.1	53.0±0.8	57.2±1.3	61.4±0.8
Normal from dominant rumpless	16	15.2±0.7	19.4±0.8	33.0±1.2	54.6±0.7	55.8±0.7	61.3±0.6
Recessive rumpless (complete or intermediate)	17	7.7±0.2	9.4±0.2	16.0±0.6	46.0±0.9	42.7±1.0	36.1±0.6
Recessive rumpless (normal caudal vertebrae)	14	7.9±0.3	9.9±0.3	18.1±0.6	52.7±0.9	56.0±0.5	58.0±0.7

TABLE 16

Percentages of ash in dry extracted bone and of calcium in ash, and of ash and calcium in terms of fresh weight of femur, tibia, and pelvis of mature cockerels from the dominant and recessive rumpless stock.

GROUP	PERCENTAGE ASH IN DRY, EXTRACTED BONE			PERCENTAGE CALCIUM IN ASH			ASH IN PERCENTAGE OF FRESH WEIGHT			CALCIUM IN PERCENTAGE OF FRESH WEIGHT		
	FEMUR	TIBIA	PELVIS	FEMUR	TIBIA	PELVIS	FEMUR	TIBIA	PELVIS	FEMUR	TIBIA	PELVIS
Dominant rumpless (complete or intermediate)	53.5 ±0.3	57.7 ±0.2	52.9 ±0.2	38.3 ±0.1	38.2 ±0.1	38.5 ±0.04	28.4 ±0.5	33.0 ±0.8	32.5 ±0.5	10.9 ±0.2	12.6 ±0.3	12.5 ±0.2
Normal from dominant rumpless	54.1 ±0.3	57.7 ±0.2	52.7 ±0.2	38.2 ±0.1	38.4 ±0.1	38.5 ±0.04	29.6 ±0.5	33.3 ±0.5	32.3 ±0.4	11.3 ±0.2	12.4 ±0.2	12.4 ±0.2
Recessive rumpless (complete or intermediate	53.6 ±0.3	57.7 ±0.2	51.6 ±0.3	38.3 ±0.1	38.5 ±0.1	38.3 ±0.1	27.9 ±0.4	30.7 ±0.8	30.3 ±0.4	10.7 ±0.2	12.5 ±0.3	11.6 ±0.2
Recessive rumpless (normal caudal vertebrae)	54.9 ±0.3	58.4 ±0.2	51.7 ±0.1	38.3 ±0.1	38.6 ±0.04	38.3 ±0.1	28.9 ±0.5	32.6 ±0.3	30.0 ±0.4	11.1 ±0.2	12.6 ±0.1	11.5 ±0.1

Further heterogeneities of considerable interest are found in relative dry weight of the bones (table 15). The following figures represent the differences between means.

	Dominant rumpless less recessive rumpless	Normal from dominant rumpless less recessive rumpless with normal caudal skeleton
Femur	7.0±1.2	1.9±1.1
Tibia	14.5±1.6	−0.2±0.9
Pelvis	25.3±1.0	3.3±0.9

It may be seen that the pelvic bones of the recessive rumpless cockerels lost much more weight during drying—that is, contained more water—than the pelvic bones of the dominant rumpless animals. A small but significant difference of the same kind was found between recessive rumpless animals with a normal caudal skeleton and normals from the dominant rumpless stock. Among the recessive rumpless animals relative dry weight of the long bones of the legs

TABLE 17

Fresh weight and dry weight in percentage of fresh weight of leg bones and pelvis of newly-hatched chicks from the dominant and recessive rumpless stock.

GROUP	NUMBER	FRESH WEIGHT IN MG.		DRY WEIGHT IN PERCENTAGE OF FRESH WEIGHT	
		LEG BONES	PELVIS	LEG BONES	PELVIS
Dominant rumpless (complete or intermediate)	48	573±8.2	637±10.5	28.3±0.2	23.3±0.2
Normal from dominant rumpless	47	591±8.2	663±10.2	28.4±0.2	23.2±0.2
Recessive rumpless (complete or intermediate)	37	452±7.9	492±11.4	29.2±0.4	24.4±0.4
Recessive rumpless (normal caudal vertebrae)	50	431±6.5	498±10.1	29.0±0.3	24.4±0.3

TABLE 18

Percentages of ash in dry extracted bone and of calcium in ash, and of ash and calcium in terms of fresh weight of leg bones and pelvis of newly-hatched chicks from the dominant and recessive rumpless stock.

GROUP	PERCENTAGE ASH IN DRY, EXTRACTED BONE		PERCENTAGE CALCIUM IN ASH		ASH IN PERCENTAGE OF FRESH WEIGHT		CALCIUM IN PERCENTAGE OF FRESH WEIGHT	
	LEG BONES	PELVIS	LEG BONES	PELVIS	LEG BONES	PELVIS	LEG BONES	PELVIS
Dominant rumpless (complete or intermediate)	33.9±0.3	25.8±0.4	36.5±0.1	35.3±0.2	9.6±0.2	6.0±0.1	3.5±0.1	2.1±0.04
Normal from dominant rumpless	34.5±0.3	25.9±0.3	36.4±0.1	35.3±0.2	9.8±0.1	6.0±0.1	3.6±0.04	2.1±0.04
Recessive rumpless (complete or intermediate)	35.4±0.3	26.5±0.4	37.0±0.2	36.8±0.1	10.4±0.2	6.5±0.2	3.8±0.1	2.4±0.1
Recessive rumpless (normal caudal vertebrae)	34.6±0.3	27.7±0.4	37.0±0.1	36.7±0.2	10.1±0.2	6.8±0.2	3.8±0.1	2.5±0.1

is much lower than that of the corresponding bones of dominant rumpless animals. No significant differences in relative dry weight exist between recessive rumpless cockerels with a normal caudal skeleton and normals which had segregated from the dominant rumpless stock.

Since the values for ash and calcium of the long bones do not differ significantly between any of the groups and since the fresh weights of the bones of the two groups of recessive rumpless birds were essentially the same, it can only be concluded that in the long bones (and pelvis) of the rumpless or inter-

mediate rumpless animals from the recessive rumpless stock, as compared with their sibs with a normal caudal skeleton, the water content was increased at the expense of organic matter. This situation finds its most extreme expression in the pelvis. It may be surmised that similar conditions prevail in the axial skeleton and that this provides the basis for the lowered resistance of the vertebral column to deformation.

It seemed important to determine if these peculiarities of bone composition are already present in newly-hatched chicks. Analyses were made of the pelvis and the pooled long bones (femur, tibia, tarsometatarsus) of one leg. The results are assembled in tables 17 and 18. Relative dry weight of the bones is essentially the same for the different groups. The ash and calcium content of the bones tends to be slightly higher in the recessive rumpless chicks than in the chicks from the dominant rumpless stock. The differences are small, and most of them are of doubtful significance. As far as they are real, they can be readily understood as an expression of the differences in body size which exist between the two stocks (OUTHOUSE and MENDEL 1933). No abnormalities of bone composition, similar to those found in adult birds, could be demonstrated by chemical analysis.

Deviations which are similar in nature to those of the adult recessive rumpless animals could, however, be established in embryos which had been treated with alizarin. The embryo material from the recessive rumpless stock was either completely or intermediate rumpless. As in all our material, the recessive rumpless animals had been derived from outcrosses to White Leghorns. The two groups of recessive rumpless embryos were 21 days of age, the White Leghorn embryos were 20 days and 6½ hours old.

Little, if any, difference in the intensity of alizarin staining could be observed in the long bones, cervical and thoracic vertebrae, and ribs of the several groups. The pelvis and the synsacral vertebrae of the recessive rumpless embryos showed a less intense alizarin staining than did the same parts of the Leghorn embryos. The same was true for the uncinate processes of the ribs, and in their instance it was possible to make a semi-quantitative estimate of the degree of calcification.

The second to sixth thoracic ribs have uncinate processes. Calcification of these processes, as of the ribs themselves, starts with the fourth rib and from there spreads in an anterior and posterior direction. The highest degree of calcification which was found in the uncinate processes of our material was assigned the arbitrary value of "1." Such an amount of calcification was generally found in the uncinate processes of the third and fourth ribs of the Leghorn embryos. Lesser degrees of calcification were expressed by fractions. The means of these estimates are given in table 19.

The close agreement between the values for the uncinate processes of corresponding ribs on the left and right side of the body and also between the two groups of embryos from recessive rumpless matings indicates a satisfactory degree of reliability of our estimates. It is evident from these observations that calcification of the uncinate processes of the recessive rumpless embryos lags far behind that of the Leghorn embryos. This difference is the

more significant since the Leghorn embryos were slightly younger than the embryos of the recessive rumpless stock. The dissimilarities of calcification in embryos of the two strains are least pronounced in the uncinate processes of the third and fourth ribs; they are most striking in the processes of the second and fifth ribs.

TABLE 19

Estimated degree of calcification in the uncinate processes of the ribs in White Leghorn and recessive rumpless embryos.

GROUP	NUMBER OF EMBRYOS	LEFT BODY SIDE, THORACIC RIB NUMBER					RIGHT BODY SIDE, THORACIC RIB NUMBER				
		6	5	4	3	2	6	5	4	3	2
White Leghorn	37	0	.83	.97	.99	.53	.03	.80	.97	.99	.47
rp2 Group I	41	0	.26	.84	.84	.17	0	.28	.83	.83	.11
rp2 Group II	38	0	.38	.82	.83	.10	0	.39	.82	.80	.14

It should be noted that the delay in calcification is confined to certain areas. It is not observable, for instance, in the tarsal bones, although the tarsalia, like the uncinate processes, were undergoing active calcification.

On the whole, then, our observations demonstrate that the recessive rumpless mutation is associated with disturbances in the chemical composition of various skeletal parts. The earliest expression of these changes was seen in late embryonic stages in which alizarin staining revealed delay in calcification of the pelvis, the synsacral vertebrae, and the uncinate processes of the ribs. In adult animals the pelvic bones showed a reduction in relative ash and calcium content, irrespective of whether or not the caudal vertebrae were deformed or lacking. The pelvic bones of animals from the recessive rumpless stock, whether actually rumpless or not, are hydrated at the expense of organic constituents. This is also true for the long bones of the legs of animals which have an abnormal caudal skeleton, but not for those with a normal tail.

This chemical picture represents a trend very similar to that found in rickets. Yet, all animals were raised on open range and had a very adequate intake of vitamin D and of calcium. There were no cases of "leg-weakness" or other symptoms of vitamin D deficiency. Fecundity and formation of egg shells were normal in the recessive rumpless females. This negative evidence does not exclude the possibility, of course, that some of the physiological events which lead to an abnormal composition of bones are common to rickets and to recessive rumplessness. In the recessive rumpless condition, however, we are not dealing with a deficiency, but presumably with an inability of the particular skeletal parts to perform all normal functions.

The association of morphogenetic abnormalities with systemic defects of structure apparently is not a common occurrence. It is of interest, therefore,

to call attention to a human case history, reported by LIEBENAM (1938) in which congenital defects of the four extremities were accompanied by widespread symptoms of osteosclerosis.

DISCUSSION

It has been demonstrated that the dominant and the recessive rumpless mutations share many features of morphological expression. There are also important differences, such as the tendency in the recessive rumpless material to show lordosis of the rudimentary caudal vertebrae or the occurrence of synsacral kypho-scoliosis and supernumerary ribs. None of these peculiarities, however, is a constant trait of the recessive rumpless animals.

A mutation which morphologically appears to have some relationship to our recessive rumpless variation has been briefly reported by CZAJA (1939). He found in a Polish breed of fowl a recessive mutation whose chief characteristic is a lumbar kypho-scoliosis. No mention is made of abnormalities of the caudal vertebrae. The scoliotic condition led in the extreme variants of this material, in contradistinction to our own, to complete immobilization of the legs. It remains unknown whether or not there is any genetic relationship between CZAJA's mutation and our recessive rumplessness.

CH'IN (1936) reported on a case of complex malformations in a human fetus (his case I) which included complete absence of the coccyx, scoliosis, bilateral asymmetry of the pelvis, fusion of ribs and spina bifida as well as horse-shoe kidney, Meckel's diverticulum, and other signs of developmental arrest. The combination of skeletal abnormalities is sufficiently similar to our material to be of interest. CH'IN'S evidence is suggestive of the conclusion that widespread arrest of development had occurred in this fetus. This is also of interest with reference to our material, since it is probable that the supernumerary ribs, frequently associated with recessive rumplessness, are likewise the result of persisting embryonic features; it is known that unspecified accidents of development may lead to the appearance of centers of ossification for lumbar ribs which otherwise would have persisted only as rudiments fused with the lateral processes of the vertebrae.

Another feature of the recessive rumpless birds which is of interest in this connection concerns the uropygial gland. These paired glands, with a single nipple, develop from two ectodermal invaginations. A doubling of the nipple has been reported as a semi-dominant mutation (HUTT 1932). Doubling of the nipples of the uropygial glands is also found in a considerable number of those recessive intermediate rumpless birds in which these glands are smaller than normal, but not in those in which the glands are formed normally, nor in the Leghorn stock from which the mutation was extracted. The doubling is apparently brought about by a developmental interference with the normal fusion of the anlage material of the nipples. This may be taken as a local effect of the intermediate rumpless condition.

It is obvious from the data which have been presented here that a series of genetic modifiers influences the morphological effects of the recessive rumpless mutation. The same was found to be true for dominant rumplessness (DUNN

and LANDAUER 1934, 1936). The expression of both mutations is affected by the sex of the individual. These influences, however, are in opposite directions. As far as the intermediate conditions of dominant rumplessness are concerned, it was shown (LANDAUER 1928) that the males tend to be more nearly normal (better differentiation of caudal vertebrae) than the females. The reverse is true in the recessive rumpless stock, for here the males show a definite trend toward more extreme abnormality.

We do not know why sex has such different effects on the expression of the two rumpless mutations. Endocrine factors are precluded by the fact that the principal features of dominant rumplessness are already determined during the third day of embryonic development, those of the recessive rumpless variation during the fourth and fifth days (ZWILLING 1942, 1945)—that is, in both instances prior to the establishment of gonadal endocrine function. It is likely that some of the genes which modify rumplessness toward normal are sex-linked and that the efficiency with which these sex-linked genes operate in the two sexes varies with the nature of the developmental processes which are affected by the two rumpless mutations.

Although the embryological evidence disposes of the possibility that secondary effects of sex play a role in modifying the recessive rumpless condition, such factors may well be instrumental in exaggerating sexual differences during later development. It is known that there is a reversal in percentage of skeletal (tibia) ash and calcium of growing chicks, the males leading at first but being overtaken by females at the age of about one month (HOLMES, PIGOTT, and MOORE 1932). This reversal is probably brought on by the emergence of a higher rate of growth in males. It is also known that cartilage calcification occurs later in males than females (HARSHAW, FRITZ, and TITUS 1934), a fact which in turn is presumably associated also with the more intense and more prolonged growth of males. The lower content of ash and calcium in the bones of males during the period of their more protracted and rapid growth may well result in more ready response to unfavorable agencies. An instance of this nature may be seen in the hereditary tendency toward deformation of the sternum described by WARREN (1937) in White Leghorn chickens. The frequency of occurrence of this abnormality and its extent were greater in males than in females.

The developmental studies of ZWILLING have shown that dominant rumplessness arises by degeneration of the presumptive tail tissues, but that recessive rumplessness is the result of abnormal formation and secondary degeneration of formed tail structures. Evidence has been presented here that recessive rumplessness is associated with disturbances in calcification of the axial skeleton and pelvis. Dominant rumplessness does not have similar effects. The evidence points to the conclusion, therefore, that the developmental mechanisms as well as the physiological effects differ in the two mutations.

It seems obvious from all observations that the abnormalities of the caudal skeleton are the principal effects produced by the recessive rumpless mutation and that the changes in other parts of the spine, in the pelvis, and the ribs are only distant repercussions. It must not be concluded, however, that these

effects are mainly or wholly indirect ones. It may well be that the same physiological agencies which disturb or prevent development of the caudal vertebrae, in other parts interfere with normal calcification of certain skeletal elements. The uncinate processes of the ribs lag in calcification from the very beginning, and there are other indications in favor of the conclusion that the proper deposition of calcium is, in certain skeletal parts, disturbed from its inception.

Kypho-scoliosis and lordosis

This brings us to a discussion of the synsacral kypho-scoliosis and caudal lordosis which are so frequently associated with recessive rumplessness. Sporadic cases of deformities of the spine have repeatedly been reported in birds (fowl: HÄRTEL 1909; CHLUMSKY 1924. Geese: SCHMIDT 1903; CHLUMSKY 1924. Ducks: CHLUMSKY 1924. *Tetrao tetrix* L.: GARBOWSKI 1896). HÄRTEL described the mechanical aspects of the standing position in fowl and concluded that the pre-pelvic muscular pull on the vertebral column is such as to predispose to kypho-scoliotic deformity. CHLUMSKY, on the other hand, emphasized that scoliosis of birds is, as a rule, congenital, that it cannot be explained as an effect of forces of gravity, but must be the result of an interaction between a skeletal abnormality and the pull of muscles and tendons. As far as the recessive rumpless material is concerned, it is known that the synsacral kypho-scoliosis is at times already established at hatching, and it is probable that this is a regular feature. The primary cause of the abnormality must be sought in a local weakness or malacia of the spine, brought out perhaps, as CHLUMSKY suggests, by the pull of muscles and tendons. It is also true, however, that no extreme forms of scoliosis were found in newly-hatched chicks, and it is quite possible that the mechanical peculiarities of the chicken skeleton, as described by HÄRTEL, tend to produce an exaggeration of the congenital scoliotic condition.

In the orthopedic, pediatric, and pathological literature the etiology of human scoliosis has been a vexing and much discussed problem. PUTSCHAR (1937) has given an excellent summary of the conflicting interpretations prevailing in this field. There is no doubt that a number of different causes may lead to scoliosis. There is also no doubt that some of these causal agencies arise by accidents of the environment, whereas others are governed by hereditary factors. The importance of heredity for the appearance of so-called "rhachitic" scoliosis has been especially emphasized by FABER (1936). But there is considerable doubt whether or not the diagnosis "rickets" is justified in all or even a majority of these cases. Many authors have called attention to the fact that frequently there are no indications of florid or healed rickets in the extremities or even in the ribs, nor a personal history which would make the earlier existence of rickets seem likely. In our own material of recessive rumpless fowl with kypho-scoliosis there are no indications whatever of a constitutional vitamin D deficiency. On the basis of similar considerations ASCHNER and ENGELMANN (1928) came to the conclusion that a familial "konstitutionelle Bereitschaft der Wirbelsäule zur Skoliose" probably accounts for many cases. Such an interpretation is supported by our evidence.

It has long been known that in man cervical scoliosis is frequently associated with the presence of "cervical ribs," and it is recognized that these supernumerary cervical ribs are not the cause of the co-existing scoliosis. Rather, it appears that the same underlying conditions predispose to both abnormalities. Similarly, it would seem that the developmental abnormalities of the axial skeleton which are associated with the recessive rumpless mutation of fowl may call forth either synsacral kypho-scoliosis or the formation of supernumerary ribs or the simultaneous existence of both abnormalities.

Our observations on rate of calcification of the uncinate processes of the ribs, the sacral vertebrae, and the pelvic bones, the fact that many of the free vertebrae show flaring epiphyseal ends, and the data on ash and calcium content of the pelvic bones of adult birds all point to developmental and physiological abnormalities of the cartilage in the axial skeleton. The resulting changes presumably leave these structures unequal to the forces of normal stress and strain. The parts which are affected most are those in which cartilage formation occurs particularly late during normal development (BLINOV 1938).

The nature of the modifiers

The caudal vertebrae of fowl are notoriously variable. Their shape and size may differ considerably from bird to bird. This is particularly true for the pygostyle, but applies also to the free caudal vertebrae. The latter, as well as the vertebral primordia which make up the pygostyle, may vary in number. On the whole, then, we have a picture which is characteristic of organs with retrogressive tendencies, and there is little doubt that this variability reflects a complex genetic situation.

It has been shown earlier that the developmental effects of the dominant rumpless mutation may be partially or even entirely suppressed by the action of genetic modifiers (DUNN and LANDAUER 1934, 1936). It was assumed that these modifications toward normal are brought about by genes which, in normal stocks of fowl, have been accumulated on account of the developmental instability of the caudal skeleton. It was further assumed that these genes serve normally to insure the complete formation of the tail.

This interpretation of the function of modifiers in terms of developmental safety has been generally accepted (DOBZHANSKY 1937; SILOW 1939). Specific objections which FISHER (1938) raised against our views have already been answered adequately by SILOW (1939) and do not need to be discussed again here. It must be said, however, that our interpretation finds strong support from ZWILLING'S (1942) embryological observations. He found that size limitation of the tail in normal embryos and complete suppression of the tail are brought about by quantitative variations of the same physiological process—namely, degeneration of presumptive tail tissue. The modifiers of dominant rumplessness reduce the amount of degeneration toward that occurring in normal embryos. These facts suggest that genes have been accumulated in normal stocks which prevent degeneration of presumptive tail tissue beyond safe limits and such genes would presumably tend to counteract—that is, modify—excessive degeneration on account of a new mutation.

The present status of our knowledge concerning genetic factors which tend to modify or prevent rumplessness in fowl is briefly as follows.

1. Dominant rumplessness may be modified toward the normal condition by multiple genes which produce their effect on heterozygous and on homozygous mutants (DUNN and LANDAUER 1934, 1936).

2. Recessive rumplessness is modified toward normal by multiple genes (evidence of the present report).

3. In experiments concerning the production of rumplessness by shaking eggs prior to incubation it was shown that the response of eggs to treatment varies with the genotype of the mothers (LANDAUER and BAUMANN 1943).

4. Rumplessness can be produced by the injection of insulin into eggs. Such experiments yield complete rumplessness and intermediate conditions. The percentage of all rumpless embryos which are obtained by this method is influenced by the genotype of the mother. The percentage of intermediate rumpless individuals differs in different stocks (LANDAUER 1945).

It may be seen, then, that genes are present in normal stocks which will prevent or suppress the production of rumplessness by genetic or extrinsic agencies. Furthermore, there are other genes which will modify rumpless development toward normal in such diverse situations as the dominant and recessive rumpless mutations or the influence of insulin.

The morphological expression of intermediate rumplessness has, in addition to many similarities, certain distinctive features in each of the three types of origin. We do not know at present if this is so because some or all of the modifiers are different in each instance or because, as in the mouse material described by DUNN (1942), identical modifiers produce unlike effects on account of dissimilarities in developmental disturbance called forth by the various rumpless-producing agencies. We may, of course, be dealing with a combination of these two eventualities.

In any case, it is evident from our observations that the primary function of the modifiers is that of protecting the organism against agencies which tend to push the course of its development into dangerous channels. Dominance of the wild type is the formal expression of these conservative and protective trends.

Since it is the function of the modifiers to reinforce normal development, one may expect that their physiological effects are mediated through normal ontogenetic processes. That this is actually so has already been discussed with reference to the modifiers of the dominant rumpless mutation. A similar situation seems to exist with regard to polydactylism of fowl (BAUMANN and LANDAUER 1944). The expression of this mutation is subject not only to general modifiers but also to regional ones whose action may be limited to one pair of extremities or to one body side These regional modifiers are susceptible to selection. There is indirect evidence suggesting that their effects are brought about by physiological processes which are associated with normal growth and differentiation of the embryo.

It may be surmised that the evidence from these divergent sources has general significance for a dynamic understanding of the action of modifying genes.

SUMMARY

A new skeletal mutation of fowl has been described which has the following principal morphological features.

The pygostyle and the free caudal vertebrae may be entirely absent; more commonly they are rudimentary and fused together into a bony knob. According to the degree of abnormality of the caudal skeleton, the uropygial gland and the tail feathers may be lacking, or the gland may be rudimentary, and there may be a reduced number of drooping tail feathers.

The intermediate conditions lead by imperceptible steps from complete rumplessness to an entirely normal tail. In all the preceding peculiarities the new mutation resembles closely the forms of expression of the dominant rumpless mutation and its modifications.

The following morphological traits distinguish the new mutation from dominant rumplessness. The rudimentary caudal vertebrae frequently have the appearance of lateral compression and show marked lordosis. In many animals there is a synsacral kypho-scoliosis of varying degree, with a compensatory thoracic or synsacro-thoracic scoliosis in the opposite direction. The pelvic bones of such animals are deformed. The acetabula stand at different levels, and this asymmetry is corrected, as far as locomotion is concerned, by coxa vara of the femur with the lower articulation. A less common part of the syndrome is the occurrence of supernumerary ribs, posterior to the last normal pair. These supernumerary ribs may be unilateral or bilateral, rudimentary or fully formed.

Sex acts as a modifier of this mutation. In males the various features of the whole syndrome tend to find more frequent and more severe expression.

The mutation is completely recessive to the normal condition. It was found in White Leghorn stock. It appears that the expression of the mutation is influenced by the action of a series of modifying genes and that the latter account for the low degree of "penetrance" and "expressivity." Some of the genetic modifiers may be sex-linked, but others and the main gene are autosomal.

Completely rumpless embryos have a slightly reduced chance of hatching; otherwise the mutation does not exert a marked effect on embryo survival. During the postnatal growth period there is a somewhat increased mortality of chicks with an abnormal caudal skeleton as compared with their sibs with normal tail vertebrae.

The ash and calcium content of the pelvic bones is reduced in all adult animals of the recessive rumpless stock, including those with normal tail vertebrae. Percentage of water in these bones is increased at the expense of organic material. This hydration is found also in the femur and tibia of rumpless and intermediate rumpless animals from the recessive rumpless stock, but not in those birds which have a normal caudal skeleton. Chemical analysis did not reveal changes of composition in the bones of newly-hatched chicks, but by alizarin staining, delayed calcification could be demonstrated in the synsacral vertebrae, the pelvis, and the uncinate processes of the ribs.

There are no signs of rickets (vitamin D deficiency), but changes in chemical composition of the axial skeleton probably account for reduced mechanical strength (lowered resistance to normal stresses), resulting in kypho-scoliosis.

There is considerable evidence that the modifiers of rumplessness are genes which serve as stabilizers of normal development of the caudal skeleton.

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LITERATURE CITED

ASCHNER, B., and G. ENGELMANN, 1928 Konstitutionspathologie in der Orthopädie. Erbbiologie des peripheren Bewegungsapparates. Berlin: J. Springer. 312 pp.

BAUMANN, L., and W. LANDAUER, 1944 On the expression of polydactylism in the wings of fowl. Anat. Rec. **90**: 225–233.

BLINOV, V. A., 1938 Zur Frage nach der Zeit der Anlagenbildung und dem Differenzierungstempo des Knorpelgewebes in verschiedenen Organen des Vogelembryos. (Russian with German summary.) Biol. Zh. (Mosc.) **7**: 311–324.

CH'IN, K. Y., 1936 A morphological study of two cases of multiple congenital malformations of the skeleton. Chinese Med. J. Supplement **1**: 82–100.

CHLUMSKY, V., 1924 Ueber die Skoliose bei Hausvögeln. Z. orthop. Chir. **44**: 470–478.

CZAJA, M., 1939 Ein semiletaler Faktor bei Hühnern, der Missbildungen der Wirbelsäule und des Beckens verursacht. Proc. Seventh World's Poult. Cong., Cleveland, Ohio, U. S. A.: p. 55.

DOBZHANSKY, T., 1937 Genetics and the Origin of Species. New York: Columbia University Press. 364 pp.

DUNN, L. C., 1925 The inheritance of rumplessness in the domestic fowl. J. Hered. **16**: 127–134.
1942 Changes in the degree of dominance of factors affecting tail-length in the house mouse. Amer. Nat. **76**: 552–569.

DUNN, L. C., and W. LANDAUER, 1934 The genetics of the rumpless fowl with evidence of a case of changing dominance. J. Genet. **29**: 217–243.

DUNN, L. C., and W. LANDAUER, 1936 Further data on genetic modification of rumplessness in the fowl. J. Genet. **33**: 401–405.

DU TOIT, J., 1913 Untersuchungen über das Synsacrum und den Schwanz von *Gallus domesticus* nebst Beobachtungen über Schwanzlosigkeit bei Kaulhühnern. Jena. Z. Naturw. **49**: 149–312.

FABER, A., 1936 Untersuchungen über die Erblichkeit der Skoliose. Arch. orthop. Unfall-Chirurgie **36**: 217–296.

FISHER, R. A., 1938 Dominance in poultry. Feathered feet, Rose comb, Internal Pigment and Pile. Proc. Roy. Soc. B **125**: 25–48.

GARBOWSKI, T., 1896 Sternosacrale Scoliose bei Rasoren und anatomische Folgen. Ann. naturh. Hofmus. **11**: 12–30.

HARSHAW, H. M., J. C. FRITZ, and H. W. TITUS, 1934 The normal development of the leg bones of chickens with respect to their ash content. J. Agric. Res. **48**: 997–1008.

HÄRTEL, F., 1909 Über die Rückgratsverkrümmung bei Tieren, insbesondere bei unseren Hausvögeln. Deuts. Z. Chir. **98**: 277–305.

HOLMES, A. D., M. G. PIGGOTT, and W. B. MOORE, 1932 The influence of sex on the size and composition of tibiae of growing chicks. Poult. Sci. **11**: 243–249.

HUTT, F. B., 1932 Eight new mutations in the domestic fowl. Proc. Sixth Int. Congress Genet. **2**: 96–97.

LANDAUER, W., 1928 The morphology of intermediate rumplessness in the fowl. J. Hered. **19**: 453–467.

1942 A new mutation of fowl affecting the axial skeleton, especially in the tail region. Genetics **27**: 152–153.

1945 Rumplessness of chicken embryos produced by the injection of insulin and other chemicals. J. Exp. Zool. **98**: 65–77.

LANDAUER, W., and L. BAUMANN, 1943 Rumplessness of chicken embryos produced by mechanical shaking of eggs prior to incubation. J. Exp. Zool. **93**: 51–74.

LANDAUER, W., and L. C. DUNN, 1925. Two types of rumplessness in domestic fowls. J. Hered. **16**: 153–160.

LIEBENAM, L., 1938 Über gleichzeitiges Vorkommen von Gliedmassenenddefekten und osteosklerotischer Systemerkrankung. Z. menschliche Vererbungs- und Konstitutionslehre **21**: 697–703.

OUTHOUSE, J., and L. B. MENDEL, 1933 The rate of growth. I. Its influence on the skeletal development of the albino rat. J. Exp. Zool. **64**: 257–285.

PROMPTOFF, A. N., 1928–1929 Inheritance of structural types in the dorsosacrum of domestic poultry. J. Genet. **20**: 29–51.

PUTSCHAR, W., 1937 Der funktionelle Skeletumbau und die sogenannten Belastungsdeformitäten. Handbuch der speziellen pathologischen Anatomie und Histologie **9**, III: 617–787. Berlin: J. Springer.

SCHMIDT, E., 1903 Zur Kenntnis der Skoliose bei Thieren. Z. orthop. Chir. **11**: 352–361..

SILOW, R. A., 1939 The genetics of leaf shape in diploid cottons and the theory of gene interaction. J. Genet. **38**: 229–276.

WARREN, D. C., 1937 Physiologic and genetic studies of crooked keels in chickens. Tech. Bull. Kans. Agric. Exp. Sta. **44**.

ZWILLING, E., 1942 The development of dominant rumplessness in chick embryos. Genetics **27**: 641–656.

1945 The embryogeny of a recessive rumpless condition of chickens. J. Exp. Zool. **99**: (in press).

INCIPIENT REPRODUCTIVE ISOLATION BETWEEN TWO SUBSPECIES OF DROSOPHILA PALLIDIPENNIS

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INTRODUCTION

THE process of transformation of races into species is in general slow. The only approach to its study is through examination of its successive stages represented on our time level in different species and species groups. Nor is the process uniform in all organisms. Even though the essential feature of speciation in sexual cross-fertilizing forms is the development of reproductive isolation between the diverging races, the isolation is attained by different means in different cases. This necessitates careful study of diverse groups of organisms, as well as of different related species with contrasting reproductive biologies, modes of life, and distributional relationships.

For many years it appeared that species of Drosophila, though they offer some of the best material for investigation of many genetic problems, are not favorable for studies on speciation; it looked as though the intraspecific variation among natural populations is very small, while the reproductive isolation between species of this genus is almost always complete. In other words, the critical stages of speciation, the intermediates between races and species, seemed to be absent, as if species formation has come to a standstill. Facts brought to light during the last decade have, however, shown that the above impressions were erroneous, based on a failure to realize that the speciation in Drosophila is frequently accompanied by very little differentiation in the visible characters. Undoubtedly separate species of this genus may be morphologically similar and even identical, while morphologically distinguishable subspecies are, compared to other organisms, rare. It is now clear that the genus Drosophila, contrary to the old idea, is quite rich in "borderline cases" between race and species. Owing to the inherent advantages of many Drosophila species as laboratory materials, they offer excellent opportunities for studies on the genetics of speciation.

In the present article we wish to report on two subspecies of *D. pallidipennis* which have developed slight morphological differences and partial hybrid sterility, without either differentiation of the gene arrangements in their chromosomes or sexual isolation. We are obligated to MRS. N. P. SIVERTZEV-DOBZHANSKY for her help in making the wing measurements, and to MISS IRENE MARKREICH for technical assistance.

MORPHOLOGICAL DIFFERENCES

Our material consisted of a strain of *D. pallidipennis pallidipennis* from Iporanga, State of São Paulo, Brazil (collected by DR. C. PAVAN), and a strain of *D. pallidipennis centralis* from Jalapa, State of Vera Cruz, Mexico (collected by G. B. MAINLAND). After the work was almost completed we obtained another

strain of *D. pallidipennis pallidipennis* collected in the city of São Paulo by DR. C. PAVAN.

The published descriptions of the two subspecies (DOBZHANSKY and PAVAN 1943; PATTERSON and MAINLAND 1944) indicate that they are very close in external appearance, as well as in anatomical and cytological features. To obtain material for detailed comparison, flies of the Iporanga *pallidipennis* and the Jalapa *centralis* strians were transferred daily to fresh culture bottles, the number of parents being adjusted to prevent overpopulation. The cultures developed in a room at 20°–22°C. When the flies hatched, they were placed to harden for a day or two in fresh bottles with food, whereupon the body length was measured in 100 freshly etherized females and as many males of each of

TABLE 1

Body and wing lengths (in mm) and the wing indices of D. pallidipennis pallidipennis and D. pallidipennis centralis.

CHARACTER	SEX	PALLIDIPENNIS		CENTRALIS	
		M±m	σ	M+m	σ
Body length	♀	4.620±0.020	0.199	4.227±0.017	0.172
"	♂	4.245±0.014	0.141	3.982±0.014	0.141
Wing length	♀	3.680±0.017	0.116	3.252±0.017	0.120
"	♂	3.386±0.017	0.121	3.220±0.017	0.122
Wing length/width index	♀	1.970±0.009	0.061	2.103±0.009	0.065
"	♂	1.936±0.009	0.064	2.059±0.010	0.072
Costal index	♀	5.132±0.050	0.347	5.338±0.045	0.311
"	♂	5.108±0.049	0.347	5.242±0.050	0.353
4th vein index	♀	1.160±0.008	0.054	1.159±0.009	0.060
"	♂	1.163±0.010	0.070	1.145±0.012	0.083
5×index	♀	0.997±0.011	0.075	1.101±0.016	0.111
"	♂	1.035±0.012	0.084	1.195±0.016	0.112

the two subspecies. Then the flies were preserved in alcohol, and served later to make the wing measurements of 50 females and 50 males of each subspecies. In making the measurements the conventions indicated in DOBZHANSKY and PAVAN 1943) were observed. In addition to the usual measurements, "maximum wing width" was taken in order to compute the wing length: wing width index; "maximum wing width" is not generally a satisfactory measurement, since it must be taken between two not very well fixed points on the wing margins; it was taken in this work because an inspection of the wings of the two subspecies suggested that they differ in the length: width ratio. A summary of the results of the measurements is given in table 1.

The data in table 1 show that *pallidipennis* is a larger fly, with an absolutely longer but relatively narrower wing, a lower costal index, and a lower 5x index than *centralis*. But none of the distinctions are sharp, and the variation distributions overlap in all cases. The 4th vein indices do not differ significantly.

If *pallidipennis* and *centralis* are raised under identical conditions, it is possible, with practice, to classify mixed lots of etherized living flies with only a few mistakes. Attempts to classify mixed lots of flies raised in mass cultures

without special efforts to make the environments uniform were less successful, although still more than half of the flies were classified correctly. No attempt to classify dried flies was made. The principal characters used in the classification are the larger body size and the more rounded (instead of parallel-sided) wing shape in *pallidipennis*. It is also possible that the eye color of young flies is a little brighter, the eyes relatively larger and the cheeks relatively narrower in *centralis*, and the mesonotum darker and suffused with a more silky sheen in *pallidipennis*. Since we studied only a single strain of each subspecies, the objection may be raised that the distinctions cited are characteristic of these strains only and not of the subspecific populations as a whole. All we can say is that an inspection of the São Paulo *pallidipennis* strain showed

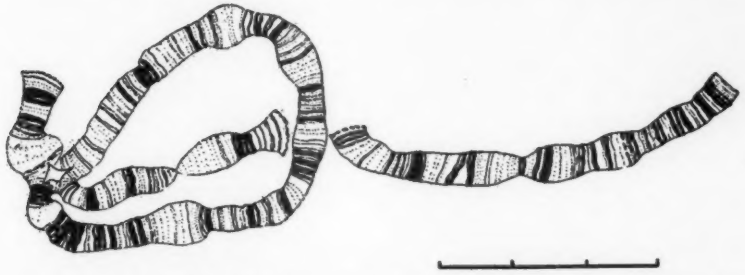

FIGURE 1.—Chromosome D in a salivary gland cell of a *D. pallidipennis pallidipennis*×*D. pallidipennis centralis* hybrid larva. The scale on the right represents 30 micra.

that it resembles the Iporanga *pallidipennis* rather than the Mexican *centralis* and that differences such as here described are seldom found between strains derived from the same population of a species of Drosophila.

CHROMOSOMES

The metaphase chromosomes of the two subspecies are similar. Salivary gland preparations were made of the F_1 hybrid larvae. Their examination disclosed that the pairing of the chromosomes in these hybrids is just as intimate as it is within either subspecies. Neither numerous unpaired sections nor the general lack of tautness, so characteristic of the salivary gland chromosomes in hybrids of species of Drosophila, were found. The only difference between the gene arrangement of *pallidipennis* and *centralis* is a fairly long inversion near the base of chromosome D found in all hybrid larvae. This inversion is shown in figure 1 (*cf.* fig. 15 in DOBZHANSKY 1944). A search for possible small rearrangements gave negative results.

Even if the above inversion should be found characteristic of the whole subspecies, and not simply of the strains at our disposal, the amount of chromosomal differentiation is still very low. Individuals heterozygous for several inversions are frequent in populations of many species of Drosophila; strains of the same species coming from regions as remote as Mexico and southern Brazil would, in general, be expected to differ in more than a single inversion.

TABLE 2

Crossability of D. pallidipennis pallidipennis (abbreviated as P) and D. pallidipennis centralis (abbreviated as C); the female parent is shown first.

	MATING	PERCENTAGE FERTILE		MATING	PERCENTAGE FERTILE
Controls	P×P	58		(PC)P)P×P	27
				(PC)C)P×P	26
	C×C	35		(CP)P)P×P	33
				(CP)C)P×P	30
P_1 crosses	P×C	76		(PC)P)P×C	38
				(PC)C)P×C	50
	C×P	35		(CP)P)P×C	34
				(CP)C)P×C	65
First backcrosses	PC×P	37		(PC)P)C×P	31
	PC×C	31		(PC)C)C×P	20
	CP×P	22		(CP)P)C×P	22
	CP×C	22		(CP)C)P×P	24
	P×PC	Sterile		(PC)P)C×C	20
	P×CP	Sterile		(PC)C)C×C	58
	C×PC	Sterile		(CP)P)C×C	18
	C×CP	Sterile		(CP)C)C×C	36
Second backcrosses	(PC)P×P	22	Third backcrosses	P×(PC)P)P	2
	(PC)C×P	25		P×(PC)C)P	Sterile
	(CP)P×P	21		P×(CP)P)P	5
	(CP)C×P	37		P×(CP)C)P	Sterile
	(PC)P×C	44		C×(PC)P)P	2
	(PC)C×C	35		C×(PC)C)P	1
	(CP)P×C	51		C×(CP)P)P	3
	(CP)C×C	46		C×(CP)C)P	Sterile
	P×(PC)P	Sterile		P×(PC)P)C	8
	P×(PC)C	5		P×(PC)C)C	6
	P×(CP)P	Sterile		P×(CP)P)C	Sterile
	P×(CP)C	1		P×(CP)C)C	10
	C×(PC)P	Sterile		C×(PC)P)C	Sterile
	C×(PC)C	Sterile		C×(PC)C)C	6
	C×(CP)P	Sterile		C×(CP)P)C	Sterile
	C×(CP)C	2		C×(CP)C)C	10

CROSSABILITY

Hybridization of *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* (denoted as P and C respectively in tables 2 and 3) succeeds without difficulty. The following technique has been evolved at the Texas laboratory to obtain quantitative data on the crossability and hybrid sterility in Drosophila crosses. Virgin females and males of the strains to be tested are selected and aged for three to five days in isolation; 132 pair matings are then made and placed in

$3\frac{3}{4} \times 1$ inch vials with food, one pair per vial. The vials are inspected on the fifth day after the mating, and any vials in which either of the prospective parents has died are discarded. On the twelfth day the vials are inspected again to record those in which offspring have and have not been produced. The number of vials among the first 100 examined which contain offspring is referred to as "percentage fertile" in table 2. It can be seen that 58 per cent of the vials contain offspring when *pallidipennis* females are crossed to *pallidipennis* males; when the same females are crossed to *centralis* males the percentage rises to 76 (the difference is probably not significant). With *centralis* females, 35 per cent of the matings produce offspring with either kind of the males.

The fecundity of the inter-racial crosses is lower than that of intra-racial matings. Fecundity is expressed as the average number of adult offspring produced by a single pair of parents under the conditions of our experiments (table 3). Approximately 35 offspring are produced by a pair of either *pallidipennis* or *centralis*. With *centralis* females crossed to *pallidipennis* males, this number falls to 16, and in the reciprocal cross it falls to only 8.

TABLE 3

Fecundity of crosses of D. pallidipennis pallidipennis and D. pallidipennis centralis (the female is shown first).

MATING	FECUNDITY
P×P	34.5
C×C	35.0
P×C	8.0
C×P	16.0
P×(PC)P)C	2.9
P×(CP)C)C	6.9
C×(PC)C)C	4.7
C×(CP)C)C	5.6

The causes of the reduction of fecundity in the inter-racial crosses are obscure. The adult F_1 hybrid flies show no indication of constitutional weakness compared to the representatives of the parental races. It is, therefore, rather improbable that a greater proportion of the hybrid than of the pure larvae succumb before reaching maturity. More likely, the lowered fecundity reflects either a lowered vitality of the sperm of one subspecies in the sperm receptacles of the other (as demonstrated in crosses of species of the *virilis* group by PATTERSON, STONE, and GRIFFEN 1942) or the delivery of fewer spermatozoa in inter-racial compared to intra-racial matings. This last possibility was suggested quite independently from the fecundity data by direct microscopic observations on the amount of sperm in the sperm receptacles of the females inseminated by males of their own or of the foreign subspecies.[1]

[1] The number of fertile eggs deposited by a female is, of course, only a fraction of the number of the spermatozoa transferred during copulation. The insemination mechanism in Drosophila is rather wasteful of spermatozoa (KAUFMANN and DEMEREC 1942). Although we did not attempt to count the spermatozoa, examination under a microscope suggested that the amounts of sperm in the receptacles vary quite considerably from female to female and that females inseminated by foreign males have fewer spermatozoa.

Observations on the amount of sperm in the receptacles were made in the course of experiments designed to test the possibility that an incipient sexual isolation may exist between the subspecies. Ten freshly hatched females of each of the two subspecies were confined with ten males of one of them for four days in $3\frac{1}{4} \times 1$ inch vials. The females were then dissected and their sperm receptacles examined for sperm under a microscope (for details of this technique see STALKER 1942, DOBZHANSKY and MAYR 1944). The results are summarized in table 4.

TABLE 4

Numbers of the females dissected and percentage of them containing sperm in experiments in which males of D. pallidipennis pallidipennis or D. pallidipennis centralis were confined with females of both subspecies.

MALES	*pallidipennis* ♀♀		*centralis* ♀♀	
	NUMBER DISSECTED	PERCENTAGE INSEMINATED	NUMBER DISSECTED	PERCENTAGE INSEMINATED
pallidipennis	106	55.7	106	33.0
centralis	111	42.3	109 *	42.2

Table 4 shows that equal proportions of females of the two subspecies are inseminated by *centralis* males; with *pallidipennis* males, a slightly but significantly higher proportion of *pallidipennis* than of *centralis* females are inseminated (the χ^2 is equal to 6.12, which, for one degree of freedom, is expected to occur by chance about once in 100 trials). Sexual isolation between the subspecies is, therefore, both weak and confined to only one of the two possible reciprocal crosses.

HYBRID STERILITY

Crosses of *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* produce hybrids of both sexes, which appear to be morphologically normal and at least equal to the parents in vigor. The F_1 hybrid males however, are, completely sterile. This has been established not only in pair matings (table 2) but also in mass cultures involving, in the aggregate, several thousand hybrid males. On the other hand, the F_1 hybrid females are fertile when backcrossed to males of either parental form. The percentage fertility tends to be lower in the F_1 hybrids than in the controls or in the P_1 crosses.

Extensive tests of the fertility of the hybrids obtained in the backcrosses have been made with the aid of the technique described above under the "Crossability" heading. Nearly 6,000 pair matings have been made for this purpose. The results are summarized in table 2. In this table the pedigree of each type of hybrid is indicated as follows. The original cross is given between parentheses, the females being always shown first. Thus (PC) is a hybrid from the cross *pallidipennis* ♀ × *centralis* ♂ and (CP) symbolizes the reciprocal cross. The male parent of the first backcross is shown next; thus, (PC)P is the offspring of an F_1 hybrid female crossed to *pallidipennis* male. The male

parent used in the second backcross is shown to the right of another parenthesis—(PC)P)P or (PC)P)C.

Females obtained in the offspring of the first backcross are fertile when backcrossed to males of either subspecies (see "Second backcrosses" in table 2); the same is true for the females obtained in the offspring of the second backcross (see "Third backcrosses" in table 2). The percentage fertility in these females is, on the average, about as high as it is in the F_1 females (see "First backcrosses" in table 2). However, the degree of fertility of the backcross females varies considerably—from 18 per cent in (CP)P)C to 65 percent in (CP)C)P. A part of this variation is doubtless due to chance, but there is a certain amount of evidence that some of the hybrid combinations are not as fertile as others, since the repetition of the crosses usually gives essentially the same results as did the original tests. It looks as though the backcross females tend to be on the average more fertile when crossed to *centralis* than when crossed to *pallidipennis* males, but this may or may not be significant.

In contrast to the complete sterility of the F_1 hybrid males, some of the males obtained in the backcrosses are fertile, although the percentage fertility is 10 or lower (table 2). It is significant that in the offspring of the second backcrosses the males derived from two backcrosses to the same subspecies are more likely to be fertile than those derived from backcrosses to different subspecies. Thus, the (CP)C)C, (CP)P)P, (PC)C)C, and (PC)P)P males are frequently fertile, while (CP)C)P, (CP)P)C, (PC)C)P, and (PC)P)C males are mostly sterile. In other words, males which carry half of the chromosomes of one subspecies and half of the chromosomes of the other are sterile, while the greater the preponderance of the chromosomes of one of the parental forms the greater the chance that a male may be fertile. This fact is compatible with the hypothesis that the sterility of the hybrids is due to interaction of several, possibly of many, genetic factors contributed by the two subspecies. The sterility of the *pallidipennis*×*centralis* hybrid males seems, therefore, to be comparable with that of the male hybrids between *D. pseudoobscura* and *D. persimilis* (DOBZHANSKY 1936).

The fecundity of the fertile males obtained in the offspring of the backcrosses is very low—such a male mated to a single female of either subspecies produces less than ten offspring on the average (table 3).

SPERMATOGENESIS IN THE STERILE MALES

Species of Drosophila having spiral testes are less favorable as material for studies on spermatogenesis than species having ellipsoid testes. *D. pallidipennis* has spiral testes which contain relatively few spermatogonia, spermatocytes, and division figures even in freshly hatched males. No detailed study of spermatogenesis has, therefore, been made either in the pure forms or in the hybrids. Nevertheless, some aceto-orcein smear preparations of the testes have revealed the essentials of the cytological situation with enough clarity.

Both subspecies of *D. pallidipennis* have five pairs of chromosomes, one of these pairs being dot-like autosomes too small to be visible in the meiotic cells. About a dozen clear diakinesis and first metaphase configurations were ob-

served in the spermatocytes of the hybrid males, and all of them showed four bivalents, just as the corresponding cells in the normal males. The first meiotic division is, nevertheless, abortive. All the chromosomes become included into a single restitution nucleus which shows the diploid number of dyads, the equational halves of which flair widely apart; no cell division takes place. The second meiotic division is apparently also abortive; at any rate, one can see many nuclei with approximately the tetraploid number of chromosomes now reduced in size. Groups of very abnormal spermatids finally degenerate to form granular masses which fill the proximal end of the testis in the hybrid males.

Normal meiotic pairing of the chromosomes followed by abortive divisions and degeneration of the spermatids may seem a surprising sequence of events. Yet, this sequence has been observed also in the hybrids between "weak" strains of *D. pseudoobscura* and *D. persimilis* (DOBZHANSKY 1934). The sterility of these latter hybrids is known to be genic rather than chromosomal; the degenerative phenomena in the spermatocytes and spermatids are not causally related to any lack of correspondence between the gene arrangements in the chromosomes of the parental forms. This is certainly true also in the *pallidipennis*×*centralis* hybrids, since in this case the parents differ in only a single inverted section (see above), which assuredly can not be responsible *per se* for the failure of spermatogenesis.

DISCUSSION

Drosophila pallidipennis pallidipennis and *D. pallidipennis centralis* are the products of a process of evolutionary divergence which seems to have reached the stage of speciation in the strict meaning of that word: these forms are on the "borderline" between race and species. They are distinguishable morphologically, at least in living flies raised under standardized conditions; yet, they show no more than a trace of the sexual isolation so characteristic for full species of Drosophila. The gene arrangements in their chromosomes differ in but a single inversion, yet the F_1 hybrid males are completely sterile and the males in the offspring of the backcrosses mostly sterile. Morphological differentiation, divergence of the gene arrangements, and the development of sexual isolation and hybrid sterility are the usual components of speciation in Drosophila. The example of *D. pallidipennis* shows that these components need not progress simultaneously.

Some closely related, though unquestionably distinct, species of Drosophila, such as *D. pseudoobscura* and *D. persimilis*, are scarcely if at all distinguishable morphologically; *pallidipennis* and *centralis* on the other hand are so distinguishable. The question may then be asked: why should the latter forms be regarded subspecies and not full species? Although it must be admitted that we are dealing here with a "borderline case," and that the information at hand is far from complete, the course adopted seems justified if systematic categories are to express the biological value of the populations concerned and not merely the degree of morphological distinctiveness. The distribution areas of *D. pseudoobscura* and *D. persimilis* broadly overlap, and yet no trace of gene exchange

has been discovered in the region in which these species are sympatric. This proves that reproductive isolation between these forms is strong enough to make their genotypes closed systems in nature. The distribution areas of *pallidipennis* and *centralis* are, unfortunately, little known. The strains at our disposal came from southern Brazil and from Mexico respectively, and no related forms have been recorded in the vast intervening territory, except perhaps DUDA's (1925) *D. hyalipennis* from Peru which appears, judging from the description, to be a fairly distinct species. Our estimate of the degree of reproductive isolation between *pallidipennis* and *centralis* rests on inference.

Laboratory experiments indicate little if any sexual isolation between *pallidipennis* and *centralis*, and no trace of inviability of their hybrids. Therefore, it is fair to assume that if populations of these forms were living side by side, numerous hybrids between them would be produced. To be sure, the propagation of these hybrids would be handicapped by the sterility of the males and the lowered fecundity of the females. However, the female hybrids are fertile enough so that the backcrosses would constitute a channel for extensive gene exchange. The lack of inversions to prevent crossing over in four out of the five large chromosomes means that gene recombinations will be formed freely. In the absence of evidence to the contrary, we believe that *pallidipennis* and *centralis* have not become reproductively isolated to an extent sufficient to maintain their genetic independence without geographical isolation. In other words, they are subspecies rather than species.

Even though *D. pallidipennis pallidipennis* and *D. pallidipennis centralis* are regarded as subspecies, the great similarity of their gene arrangements makes them the most extreme case known in Drosophila of a failure of chromosomal differentiation to accompany the genetic divergence which apparently leads to speciation. This raises the general problem of the role played by chromosomal differentiation in evolution.

SUMMARY

Drosophila pallidipennis pallidipennis and *D. pallidipennis centralis* are known to occur in southern Brazil and in Mexico respectively. The strains examined show slight morphological differences sufficient to distinguish the subspecies in the living material raised under standardized conditions but probably not in dried specimens raised in different environments.

The gene arrangements in the chromosomes of the two subspecies are identical, except for an inversion in one of the autosomes. The pairing of the homologous chromosomes in the salivary gland cells of the hybrids is as intimate as it is in the parental forms. The subspecies cross readily and produce fully viable hybrids. No sexual isolation is apparent. The F_1 hybrid males are completely sterile, but the females are fertile. Females obtained in the offspring of the backcrosses are fertile. Some of the males obtained in the offspring of the first backcross are fertile, and the proportion of fertile males increases in the offspring of the second backcross. Taken as a whole, the data suggest that the sterility of the hybrids is caused by interaction of several, perhaps of many,

genes contributed by the parental forms. Gross disturbances are apparent in spermatogenesis of the sterile hybrid males, although the meiotic chromosome pairing seems to be normal.

The combination of a slight but perceptible morphological differentiation with the near-identity of the gene arrangement, and of the lack of sexual isolation with hybrid sterility suggests that the different components of the process of speciation characteristic for the genus Drosophila are largely independent.

LITERATURE CITED

DOBZHANSKY, TH., 1934 Studies on hybrid sterility. I. Spermatogenesis in pure and hybrid *Drosophila pseudoobscura*. Z. Zellf. mikr. Anat. **21**: 169–223.

1936 Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hydrids. Genetics **21**: 113–135.

1944 Distribution of heterochromatin in the chromosomes of *Drosophila pallidipennis*. Amer. Nat. **78**: 193–213.

DOBZHANSKY, TH., and E. MAYR, 1944 Experiments on sexual isolation in Drosophila. I. Geographic strains of *Drosophila willistoni*. Proc. Nat. Acad. Sci. **30**: 238–244.

DOBZHANSKY, TH., and C. PAVAN, 1943 Studies on Brazilian species of Drosophila. Bol. Facul. Fil. Cien. e Letr. Univ. São Paulo **36** (Biologia Geral No. 4): 7–72.

KAUFMANN, B. P., and M. DEMEREC, 1942 Utilization of sperm by the female *Drosophila melanogaster*. Amer. Nat. **76**: 445–469.

PATTERSON, J. T., and G. B. MAINLAND, 1944 The Drosophilidae of Mexico. Univ. Texas Publ., **4445**: 9–101.

PATTERSON, J. T., W. S. STONE, and A. B. GRIFFEN, 1942 Genetic and cytological analysis of the *virilis* species group. Univ. Texas Publ. **4228**: 162–200.

STALKER, H. D., 1942 Sexual isolation studies in the species complex *Drosophila virilis*. Genetics **27**: 238–257.

IMMUNOGENETIC STUDIES OF CELLULAR ANTIGENS: INDIVIDUAL DIFFERENCES BETWEEN SPECIES HYBRIDS[1]

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PREVIOUS papers from this laboratory have dealt with segregation of antigenic characters of the red blood cells, peculiar to one or the other of the parental species, in hybrids from matings between Pearlneck (*Streptopelia chinensis*) and Ring dove (*St. risoria*), Pearlneck and Senegal (*St. senegalensis*), and others. The use of the immunological technics described in these papers gave results which allow the conclusion that, in a comparison of these pairs of species, each possesses two kinds of cellular antigens—namely, (a) those antigens which are *common* to both species and (b) those which are *specific* to each. Indeed, similar comparisons between several species of the genus Columba have given results which make it plausible to state that parallel conclusions may be made in the comparison of any two species, whether or not they may hybridize. References to the pertinent papers have been given in a recent review (IRWIN and CUMLEY 1943).

Many if not all of the cellular antigens which differentiate Pearlneck from Ring dove have been obtained in unit form (IRWIN 1939). Furthermore, both genetic and immunological tests coincide in showing that, of the nine or ten cellular characters specific to Pearlneck (d-1, d-2, d-3, d-4, d-5, d-6, d-7, d-4.d-8, d-9 and d-11), Senegal shares with Pearlneck all of d-1, d-2, d-3, d-4, d-5, d-4.d-8, d-9, but only a part of d-6, d-11 and of one or more others (IRWIN and COLE 1940). That is, several characters specific to Pearlneck, in comparison with Ring dove, are also specific to Senegal and represent antigens shared by the two species to the mutual exclusion of Ring dove.

Hybrids have now been obtained between Senegal and Ring dove, and backcross offspring are being produced in matings to the latter. The cells of the species hybrids have been tested for differentiation of the individuals and in relation to the specific characters of Pearlneck. The antigenic differences between these species hybrids are considered in this paper. In comparison, individual differences in antigens peculiar to Pearlneck among the hybrids from the cross of Pearlneck and Ring dove have been rarely found (IRWIN and COLE 1936; and unpublished data on other hybrids of this cross).

MATERIALS AND METHODS

Several species hybrids have been obtained in two matings (D376 and E153) of Senegal males to Ring doves. Only one hybrid ($E27R_2$ in table 1) has been produced in a reciprocal mating. These matings were made under the super-

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vision of one of us (L.J.C.); the immunological tests have been done by the other (M.R.I.). The hybrids were of both sexes in approximately equal numbers, as was also noted in the hybrids between Pearlneck and Ring dove.

The immunological technics employed in this work have been described in detail in preceding papers (IRWIN and COLE 1936; IRWIN 1939). Except for the fact that Senegal antiserum has been used in these tests, as well as anti-Perlneck serum, the general procedures of antibody production, agglutinin-absorption, and the agglutination tests are reasonably well standardized, as previously described, and need not be repeated here.

EXPERIMENTAL RESULTS

The antiserums to Senegal were absorbed by Ring dove cells and anti-Ring dove serum by Senegal cells, and these reagents were used in testing the agglutinability of the corpuscles of Ring dove, Senegal, and the species hybrids (F_1 hybrids). The details of these trials will not be given here, but will be supplied if requested. As a result of these various tests, it can be stated that the cells of both Ring dove and Senegal possess antigens peculiar to each, and part or parts of at least some of the characters peculiar to each species are found in the cells of the species hybrids. The three classes of cells were agglutinated equally by the unabsorbed antiserums, indicating that the antigens held in common were present in sufficient quantity to prohibit any differentiation of the species without antibody absorption. The relationships of the species-specific and *common* substances for Senegal, Ring dove, and their hybrids could be represented, with but little if any change, by the diagram in a preceding paper (IRWIN and COLE 1936) which depicts the antigens of the cells of Pearlneck, Ring dove, and their hybrids.

However, the hybrids between Senegal and Ring dove differ from those between Pearlneck and Ring dove in that they possess very little, if any, new or hybrid substance which distinguishes them from their parental species. That is, none of several antiserums to these hybrid cells has reacted with the hybrid corpuscles following absorption with those of both Senegal and Ring dove. However, very recent results have suggested that these hybrids may have a minute amount of a new or hybrid substance. These will be presented elsewhere.

But when the cells of individual species hybrids were used with those of Ring doves in absorption of Senegal antiserum, not only were there antibodies remaining in the test fluids for Senegal corpuscles but also for those of some of the other hybrids. These reactions are summarized in table 1. All or nearly all these tests have been made at least twice, some repeatedly over a period of years, using reagents from independent absorptions and cells from separate bleedings. Duplicate tests of many combinations of reagents and cells have also been done with test fluids from different antiserums to Senegal. With but few exceptions, the readings have been repeatable.

The amount of red blood cells obtainable from a single hybrid was usually about 0.8 cc to 1.5 cc. The reagent or test fluid prepared by the use of such cells in absorption was not sufficient to allow tests to be made with the cells of

many other birds at any one time. For example, if 1 cc of packed cells was obtained from a hybrid, only about 0.75 cc of a reagent (Senegal antiserum absorbed with cells of both Ring dove and the species hybrid) could be prepared from it. This would be sufficient to determine the agglutination of the cells of only seven or eight birds in all, including the two kinds of absorbing cells as negative controls and those of Senegal as a positive control, since 0.1 cc of reagent was required for each such mixture of reagent and cells. The ideal setup of reading the agglutination of the cells of all the hybrids at a given time was rarely possible. Thus, less rigid requirements than ordinarily should prevail may be permitted in view of the agreement of reactions of cells and reagents at different times.

The table lists a few readings which were negative (0) at one time and positive (± or +) at another. Most, if not all, such significant deviations have been noted in the reactions of reagents obtained from different antiserums, not in those prepared from a single antiserum at different times. Each antiserum may be said to have its own individuality in antibody content, and one antiserum may contain an antibody for a particular antigen while another does not (IRWIN and GOLDEN 1942), so that complete agreement in reactivity between different antiserums to the same antigens should not always be expected. Pertinent examples showing that antibodies to a (presumed) single antigenic character, the $\overline{M}$ or $\overline{N}$ of human beings, may vary in different antiserums are given by LANDSTEINER and LEVINE (1928), LANDSTEINER and WIENER (1937), and by WIENER (1938).

It may be noted in table 1 that there are a few individuals which gave equivalent reactions and which may therefore be said to have equivalent antigens of Senegal. That is, the cells of each removed antibodies for the other, and the "reagents" prepared by using the cells of each reacted similarly with all other cells tested. Also, any cells equivalent by this criterion should react alike with all other reagents. For example, within family D376, birds C, F, L, U, and probably P, fulfill these requirements of antigenic equivalence, each to the others, with but few discrepant reactions. The discrepancies were primarily weak reactions at one time or another of the cells of members of this group with reagents made from D376L or M, whereas ordinarily no reactions at all were noted (see table 1). These five hybrids are put into group 1 for ease of designation in this paper. Another such class is made up of D376D, K and M, although the reagents prepared by absorption with the cells of these respective hybrids did not always react alike with other cells, principally the reagent for D376M with the cells of birds of group 1. Despite possible differences between these three hybrids, they are classed as being antigenically equivalent, and make up group 2. The reagents made from the cells of the respective members of group 1 agglutinated the cells of each of the three hybrids of group 2, but those of the latter class removed all or nearly all the antibodies for the several birds of the first. Hence, the inference seems reasonable that the hybrids of group 2 contained at least one more antigen particular to Senegal than did those of group 1.

The remaining four hybrids of this family, D376H, V, W and A_2, appear to

TABLE 1

Tests for individuality in the cellular antigens of hybrids between Senegal and Ring dove.

CELLS ↓	REAGENTS PREPARED FROM SENEGAL ANTISERUM ABSORBED BY A COMBINATION OF CELLS OF RING DOVE AND THOSE OF THE INDIVIDUAL HYBRIDS AS LISTED: HYBRIDS OF FAMILY D376												E27	HYBRIDS OF FAMILY E153							
	C	D	F	H	K	L	M	P	U	V	W	A_2	R_2	S	U	V	W	Y	Z	C_2	D_2	F_2
Ring dove	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Senegal	+	+	②, +	±	+	②, +	+	+	②, +	+	+	+	+	+	+	+	+	+	+	+	+	+
D376C	o	o	o	+	②	o, ±	o, ±	②	o, ?	+	+	+	+	+	+	+	+	+	+	+	+	+
D376D	±	o	±	+	o	?, +	o	+	+	+	+	+										
D376F	o	o	o	+	②	o	o	②	o	+	+	+										
D376H	+	+	+	o	+	+	+	+	+	o, ?	o, ?	o										
D376K	±	o	?, +	+	o	±	o	+	+	+	+	+										
D376L	o	o	o	+	②	o	o, ±	②	o, ?	+	+	+	+	+	+	+	+	+	+	+	+	+
D376M	+	o	?, +	+	o	?, +	o	+	±	o, +	+	+	+	+	+	?, ±	+	?	+	+	②, ?	+
D376P	②	o	o	+	②	②	②	o	o	+	+	+										
D376U	o	o	o	+	②	o	o, ±	②	o	+	+	+	+	+	+	?, ±	+	±	+	+	②, +	+
D376V	+	o, ±	±	?, ±	?	?, ±	?, ±	±	±	o	o	o, +	+	+	+	?, ±	+	+	+	+	?	+
D376W	±	±	±	?	?	±	+	±	+	o	o	?, ±										
$D376A_2$	±	o, +	±	o	±	±	②, ±	±	+	o, +	o, ±	o										
$E27R_2$	+					+	o, ±		+	②, ±			o	+	+	②, ?	②, ±	②	+	+	②	+
E153S	±					+	?		±	②, ?			?	o	②, ?	o, ±	?, ±	?	o, ?	o	?	o
E153U	±					±	?		±	?			?	o	o, ?	±	?, ±	?	o	o	?	o
E153V	+					+	±		+	②			+	+	+	o, ?	+	?	+	+	②	+
E153W	+					+	±		+	②			②, ?	+	+	②	o	o, ±	+	±	②	±
E153Y	+					+	o		+	②			+	+	+	o	+	o	+	+	o	+
E153Z	o, ±					?, ±	o		?, ±	②			②	o	o, ?	②	②, ?	②	o	o	②	o, ±
$E153C_2$	o, ±					②, +	o		?	?			②	o	o	②	②, ?	②	o	o	②	o
$E153D_2$	+					+	+		+	±			+	+	+	±	+	?	+	+	o	+
$E153F_2$	+					+	+		±	±			±	o, ?	②, ?	±	?	?	②, ±	o, ?	?	o
Tests with Pearlneck cells and with cells carrying unit-characters of Pearlneck:																						
Pearlneck	+	+	+	+	+	±	o, +	+	±	o, +	+	+	±	+	+	o	+	o	+		②	+
d-1	+					+	o		+	o			o	+	+	o	o	o	+		o	+
d-2	o					o	o		o, ?	o			o	+	±	o, ±	+	o	+		o	+
d-4	o					o	o		o	o			o, ?	+	±	±						

Symbols: + = strong agglutination of various degrees; ± = definite but weak agglutination; ? = very weak agglutination, if any; o = no agglutination—at the first dilution of serum; ② = no agglutination at the second dilution of serum.

form still another group, possibly two. The cells of D376H and A_2 seem to be equivalent antigenically, differing only slightly from those of V and W.

These individual differences are most readily explained by assuming heterozygosity of the Senegal parent of the hybrids of this family in one or more chromosomes bearing a gene or genes for characters specific to the species. And since it has been shown (IRWIN and COLE 1940) that Senegal cells carry a part or all of each of the known unit-characters of Pearlneck, in contrast with Ring dove, means are at hand to test whether this segregation was by virtue of antigens *shared* by Pearlneck and Senegal. Accordingly, many of these reagents were tested with cells carrying the respective unit-antigens of Pearlneck.

These tests were made at two different times, using one Senegal antiserum at one time and another at the second. Antibodies for the d-1, d-2, d-4, d-5, d-7, and d-11 antigens of Pearlneck were present in one or both of the antiserums used, although the antibodies to the d-4 and d-7 substances were only weakly reactive (The d-4 and d-9 antigens are not considered as separate entities in this report.) Since antibodies for all antigens of Pearlneck except d-1, d-2, and d-4 were removed from the Senegal antiserum by absorption with the combination of cells of Ring dove and the respective hybrids of family D376 then available, only the reactions of the various test-fluids with these three antigens are given in table 1.

It will be noted in the table that, except for discrepancies in tests with reagents made from cells of D376M and V, each of these reagents reacted definitely with the cells of Pearlneck. Also, the reagents for D376C, L and U agglutinated the cells containing the d-1 character, but not those containing d-2 or d-4. That is, these three hybrids carried antigens d-2 and d-4—at least to the extent that antibodies specific to them were present in the immune serums—but not d-1. But the cells of D376M and V removed antibodies not only for d-2 and d-4 but for d-1 as well. Thus, the antigenic difference between the cells of group 1 consisting of D376C, L and U available for these tests, and those of group 2 (as D376M) are readily explainable. Birds of group 2 (as D376M) carry antigens d-1, d-2, and d-4, while those of group 1 (as D376C, L and U) carry only d-2 and d-4. The proposal stated above, that the cells of group 2 (as D376M) have at least one antigen more than those of group 1 (as D376C) is thereby substantiated. Unfortunately, the antigenic differences existing between individuals of these two groups (1 and 2) and D376V are not resolvable in terms of known unit-substances. D376V appears to possess both d-1 and d-2, but birds of groups 1 and 2 have at least one, possibly the same, antigen not present in D376V, and D376V has at least one substance not present in hybrids of groups 1 and 2.

From these results it seems certain that the Senegal parent of this family was heterozygous for at least two pairs of chromosomes, possibly three, bearing genes for cellular characters specific to Senegal. One of these carried a gene or genes for an antigen presumably homologous with the d-1 of Pearlneck. The one or more other pairs were heterozygous for genes producing the cellular characters which differentiated the cells of D376H and A_2 from those of groups 1 and 2, respectively, and vice versa.

Individual variation in the antigenic structure of cells of the species hybrids in family E153 may also be deduced from the reactions summarized in table 1. It appears that the cells of E153S, U, Z, C_2, and possibly of F_2, were equivalent antigenically, according to the criteria stated earlier. Likewise, the antigens of E153V and Y, and possibly D_2, are more or less alike, with those of W being different from the others. No individuals of this hybrid family had antigens equivalent to those of any bird in family D376. However, hybrids of two antigenic groups in family D376 (M and V) seemingly contained all the antigens of some hybrids of the E153 family, since absorptions by the cells of either D376M or V removed antibodies for the cells of some—but not necessarily the same—hybrids of family E153.

When the cells of birds within family E153 were used in tests of the presence or absence of specific Pearlneck antigens, it was found that E153S, U, Z, and F_2 lacked d-1, d-2 and possibly d-4 (see table 1). That is, the reagents made by using both these and Ring dove cells in absorption of anti-Senegal serum reacted definitely with cells containing any one of these three antigens of Pearlneck. By the same method of analysis, E153W possessed d-1 but not d-2, while E153V, Y, and D_2 contained both d-1 and d-2. Hence it may be concluded that the Senegal parent of the E153 family was heterozygous for a minimum of two pairs of chromosomes—that is, those producing d-1 and d-2, respectively.

It is not intended in this paper to attempt a genetic interpretation of all the antigenic differences noted among the species hybrids of either family, but rather to show that at least some of these differences are explainable by a segregation of genetic characters already known in another species.

The results stated above were tested further by the use of Pearlneck antiserum in reactions comparable to those summarized in table 1. That is, Pearlneck antiserum was absorbed by the combination of the cells of Ring dove and a species hybrid from the Senegal Ring dove cross, and these reagents were then tested for reactions with the cells of several unit antigens of Pearlneck. The results obtained are summarized in table 2. Corpuscles containing antigens d-6 and d-11 were not used in these tests, for the cells of Senegal do not contain more than a minute amount of these two antigens (IRWIN and COLE 1940). Hence, there would be only positive reactions of any reagents of table 2 with cells containing either of these antigens of Pearlneck, even if an antigen similar to either were segregating in the species hybrids.

One main difference may be noted in the reactions summarized in the tables. From those given in table 1, it was concluded that D376C, L, M, U and V, $E27R_2$, E153V, Y, and D_2 contained all of antigen d-2. But in table 2, the cells containing d-2 were agglutinated by all the reagents listed in the table, including those made by the use in absorption of the cells of seven of the above nine hybrids. Unless the antibodies for the d-2 character, or for other known characters, are different in the antiserums to Pearlneck and Senegal, respectively, the reactions given in the two tables toward the unit-substances of Pearlneck should be the same. Since they are different, primarily as regards the d-2 char-

acter, it may be inferred that the d-2 character was not the same in the two species.

Prior to making these tests, any absorption of other Pearlneck antiserums with Ring dove and Senegal cells, or with those of Senegal only, had usually removed antibodies for the d-2 antigen (IRWIN and COLE 1940). But with the particular Pearlneck antiserum (163S1) used in producing the reactions given

TABLE 2

Tests of the cells of species hybrids between Senegal and Ring dove for their content of unit antigenic characters particular to Pearlneck, as compared to Ring dove.

	CELLS ↓	REAGENTS PREPARED BY ABSORBING PEARLNECK ANTISERUM WITH RING DOVE CELLS ONLY, OR IN COMBINATION WITH THOSE OF A HYBRID BETWEEN SENEGAL AND RING DOVE										
		RING DOVE	D376				E27	E153				
			C	L	M	U	R_2	S	U	V	Z	D_2
	Ring dove	o	o	o	o	o	o	o	o	o	o	o
	Senegal	C	++	++	++	++	+	++	++	±	++	±
Unit characters of Pearlneck	d-1	++	+	+	o, ?	+	?	+	+	o	+	o, ±
	d-2	++	+	+	+	+	+	+	+	±	+	+
	d-3	+	o	o	o	o	o, +	o	o	o	o	o
	d-4	+	o	o	o	o	+	±	±	±	+	±
	d-5	++	②	②	o	②	o	o	o	o	o	o
	d-7	++	o	?	o	o	o	o	o	o	?	o
	d-12	+	o	o	o	o	o	o	o	o	o	o
	D376C		o	o	o	o	+			±		
	D376L			o								o
	D376M		+		o							
	D376U					o						
	$E27R_2$						②, ?					
	E153S		?	o	?	?	o	o	o	o	o	o
	E153U						o	o	o		o	o
	E153V									o		o
	E153Z		o	?	o	?	o	o	o	o	o	
	$E153D_2$		+	+	±	+				o		o

Symbols: C=complete agglutination; ++=strong agglutination; +, ±, ?, o and ② as in table 1.

in table 2, it was found that Senegal cells did not absorb all the antibodies for the d-2 character. What now appears to be the most reasonable explanation of the relationship of the d-2 character in Pearlneck and Senegal is as follows. A part of the d-2 component, which has behaved as a unit in backcrosses to Ring dove (IRWIN 1939), is shared with Senegal, but another part is specific to Pearlneck. Some Pearlneck antiserums contained antibodies primarily if not entirely for that fraction of the d-2 character which is shared between Pearlneck

and Senegal. Only when an antiserum to Pearlneck contains antibodies to that part of the d-2 antigen which is peculiar to Pearlneck, as compared to both Senegal and Ring dove, can this part be detected. Obviously, Senegal antiserums would contain antibodies for only that part of the d-2 antigen which was common to the two species.

Other than revealing that the d-2 substance is not present *in toto* in Senegal, an analysis of the reactions given in table 2 agrees with the previous finding that all of the d-1, d-3, d-4, d-5 and d-7 antigens of Pearlneck are shared with Senegal. Thus the cells of D376M removed all, or nearly all, the antibodies from Pearlneck antiserum for d-1, as did also those of $E27R_2$, E153V, and D_2. The four hybrids of the D376 family presumably carried the d-4 antigen; the five tested of the E153 family did not. This latter statement has been verified by the use of a reagent which contained antibodies only for the d-4 antigen. Agglutination occurred in a mixture of this reagent with the cells of each of two birds of family D376 (C and L), but not with any of four tested of family E153 (S, U, D_2, and F_2), showing, in an independent test, that D376C and L both carried d-4, while the other four hybrids did not.

DISCUSSION

The segregation observed in the species-hybrids between Senegal and Ring dove of two characters specific to Pearlneck—d-1 and d-2—and the presence of d-4 in several hybrids of one family (D376), as compared with its absence in another (E153), mean that individual differences must occur among Senegals in one or more of these three antigens. Variations among Senegals in the presence or absence of a part or all of the d-1 and d-4 characters have actually been noted by the use of special reagents; no such individual differences have been observed in tests on a limited number of Pearlnecks.

Whether such variation has any significance in the differentiation of Senegal and Ring dove by means of antigens is unknown at the present writing. It is conceivable that Senegals lacking d-1, for example, may have a gene or genes for an alternative character to d-1 which likewise is specific to the species. On the other hand, it is possible that in Senegal the alleles of the gene or genes producing any one of these three substances may be homologous to one or more in Ring dove—that is, the antigenic effects are common to the two species. Certainly, if the latter situation prevailed, the number of chromosomes in Senegal carrying genes producing species-specific characters of the species could be reduced by one, two or three, but the species would still be distinct from Ring dove by virtue of other antigens, and by other criteria. Naturally the antigens of the serum, which appear to be genetically independent from those of the cells, would still play their role in species differentiation (CUMLEY *et al.* 1943), as probably would other genic effects, as yet unknown.

The individual differences noted among these hybrids, however, have not yet shown that any one of the three segregating antigens is due to a single entity in Senegal, as each appears to be in Pearlneck. Considerable progress has been made in obtaining the cellular characters specific to Senegal as units

in backcross hybrids. Tests on the segregation of antigens peculiar to Senegal, and their interrelationships with those of Pearlneck, will be reported in subsequent papers.

SUMMARY

Individual differences in antigenic content were observed among the cells of species hybrids of the cross of Senegal × Ring dove. It was found that some of these differences could be explained by the segregation of at least a part of three separate antigenic characters (d-1, d-2, and d-4) peculiar to Pearlneck—as compared with Ring dove—which are also shared by Pearlneck, in whole or in part, with Senegal. Differences in reactivity of reagents prepared from Pearlneck and Senegal antiserums, respectively, showed that a part of the d-2 substance of Pearlneck is shared by Pearlneck and Senegal, but a part is particular to Pearlneck.

LITERATURE CITED

CUMLEY, R. W., M. R. IRWIN and L. J. COLE, 1943 Genic control of species-specific antigens of serum. J. Immunol. **47**: 35–51.

IRWIN, M. R., 1939 A genetic analysis of species differences in Columbidae. Genetics **24**: 709–721.

IRWIN, M. R., and L. J. COLE, 1936 Immunogenetic studies of species and of species hybrids in doves, and the separation of species-specific substances in the backcross. J. Exp. Zool. **73**: 85–108.

IRWIN, M. R., and L. J. COLE, 1940 Further studies of the interrelationship of cellular characters in Columbidae. Genetics **25**: 326–336.

IRWIN, M. R., and R. W. CUMLEY, 1943 Immunogenetic studies of species relationships. Amer. Nat. **77**: 211–233.

IRWIN, M. R., and ALFRED GOLDEN, 1942 Individual response in rabbits to immunization with a complex antigen. J. Infect. Dis. **70**: 119–125.

LANDSTEINER, K., and P. LEVINE, 1928 On individual differences in human blood. J. Exp. Med. **47**: 757–775.

LANDSTEINER, K., and A. S. WIENER, 1937 On the presence of M agglutinogens in the blood of monkeys. J. Immunol. **33**: 19–25.

WIENER, A. S., 1938 The agglutinogens M and N in anthropoid apes. J. Immunol. **34**: 11–18.

INHERITANCE IN NICOTIANA TABACUM. XIX. IDENTIFICATION OF THE TABACUM CHROMOSOME REPLACED BY ONE FROM N. GLUTINOSA IN MOSAIC-RESISTANT HOLMES SAMSOUN TOBACCO

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IN ORDER to produce better cultivated plants and animals by hydridization, the breeder usually relies upon an exchange of genic material between varieties or species. This is accomplished by conjugation of the chromosomes in pairs during meiosis and subsequent crossing over. In interspecific hybrids, however, chromosomes frequently do not conjugate. Therefore the utilization of valuable genic material in species closely related to a particular crop is limited by the ability of their chromosomes to pair in the hybrid.

Hybrids between wheat and rye are a case in point. Though they have often been produced, only rarely has a stable strain of wheat with one or more rye characters been secured. BLEIER (1933) and LEDINGHAM and THOMPSON (1938), among others, have obtained negative results. These failures presumably are due to the infrequent pairing of chromosomes in the meioses of the hybrids: most investigators found that the modal number of pairs ranged from zero to two (reviewed by BLEIER 1934 and by O'MARA 1940). There are instances, however, in which stable wheat strains with rye traits have been obtained. In those reported, this was accomplished through the introduction of a pair of rye chromosomes into the wheat complement. KATTERMANN (1937, 1938) thus produced strains of wheat with the rye character "hairy neck." Cytological studies showed his plants to have 20 pairs of wheat chromosomes and one pair of rye. Meiosis was regular, with 21 pairs in 94 percent and nonconjunction in only two per cent of more than 900 cells studied. KATTERMANN introduced the term *Substitutionsbastard* to designate such a type. However, only organisms having some degree of heterozygosis should be called "hybrids." In the following the term *alien subbstitution race* is being used as proposed by PROFESSOR R. E. CLAUSEN. Another case of substitution is reported from Avena, where NISHIYAMA (1939) replaced a pair of chromosomes from *A. fatua* by a pair from *A. barbata*.

A similar substitution race has been obtained in Nicotiana. HOLMES (1938) derived a mosaic resistant strain[1] of *N. Tabacum* from a *N. Tabacum*×*glutinosa* amphidiploid (named "Holmes Samsoun"). The author, in an earlier paper in this series (1943), showed that this was accomplished by the substitution of a pair of *N. glutinosa* chromosomes carrying the resistance factor for a *Tabacum* pair in the tobacco genome. In the present paper the identity of the replaced *Tabacum* chromosome will be demonstrated.

[1] The leaves and flowers of *N. Tabacum*, upon inoculation with the common mosaic virus, show mottling and distortion, while *N. glutinosa* restricts the virus to localized necrotic lesions. These symptoms will be referred to as "mottling" and "necrotic" throughout this paper.

METHODS AND RESULTS

The series of monosomics of the Purpurea variety of *N. Tabacum* established in this laboratory (CLAUSEN and CAMERON 1944) provided a tool for establishing which pair of *Tabacum* chromosomes had been replaced. It seemed feasible to identify the replaced chromosome by comparing plants carrying it in a monosomic condition with the types in the collection.

Monosomics were established by backcrossing F_1 (Purpurea×Holmes Samsoun) to Purpurea, using the hybrid as the female parent. The constant failure of a *Tabacum* chromosome from Purpurea to pair with the *glutinosa* substitute from Holmes Samsoun in the F_1—this failure had led to the detection of the substitution—and subsequent lagging of the two univalents gave a little less than 60 per cent monosomics in the backcross populations (table 1). The characteristics of these monosomic plants were compared with the various types of Purpurea.

TABLE 1

Proportion of monosomics in backcross populations from F_1 Purpurea×Holmes Samsoun to Purpurea.

POPULATION NO.	MONOSOMICS	TOTAL	% MONOSOMICS
41 B	66	112	58.9
42 388	24	46	52.2
Total	90	158	57.0

The monosome

The observed monosome was of medium large size; since the centromere was rarely evident, it usually appeared at meiotic metaphase as an ellipsoid or almost round body (fig. 1) While these observations did not suffice to establish the identity of the monosome or even to narrow the possibilities to a very few, they did eliminate more than one-half of the 24 types from consideration.

Plant morphology

It takes close acquaintance with all the monosomics to identify a particular one. Dr. CLAUSEN was kind enough to compare the monosomics obtained from the backcross of the F_1 with those of Purpurea in his collection. It should be borne in mind that this backcross population was heterozygous for many allelic pairs derived from Purpurea and Samsoun (the tobacco ancestor of Holmes Samsoun). This heterozygosity made the comparison with the pure strain Purpurea monosomics difficult. However, the backcross monosomics could be identified as resembling most closely the haplo-H type, and it will be shown below that this identification was correct.

Pollen characteristics

Pollen preparations of single anthers in lactophenol stained with acid fuchsin were used to compare the degree of pollen abortion of the monosomics derived

from Holmes Samsoun with that of the pure strain Purpurea haplo-H type. Such counts proved to be highly dependent upon a personal equation and the results cannot, therefore, be compared with the data given by CLAUSEN and CAMERON (1944) for haplo-H. While the pure strain monosomics were found to have about 50 per cent abnormal grains, the percentage for the backcross monosomics was about 65 per cent (table 2). While these frequencies are not identical, it must be taken into account that the observations were made in different years. Furthermore, one count was made from a single highly homozygous plant, while the other represented an average from a large number of backcross plants.

TABLE 2

Comparison of pollen counts of haplo-H and the monosomic Holmes Samsoun derivative.

CROSS	LOCATION	SAMPLE	% ABNORMAL GRAINS
Purpurea haplo-H×Purpurea Plant 44 208p26	Field	673 pollen grains	52.0
(Purpurea×Holmes Samsoun) ×Pupurea—Population 41B	Greenhouse	Mean from 66 plants	66.0

The pollen samples from the two types were similar in that there was a gradation in pollen size from fully aborted to normal. According to CLAUSEN and CAMERON (1944), this is characteristic of monosomics A, B, C, D, E, H, and S. Of these B, C, and E could be eliminated from consideration because of their small monosomes, A and D because of their extremely low pollen abortion, leaving only H and S. Since the S type has a sufficiently distinct flower morphology and ovular transmission (about 30 per cent, see below) it may also be disregarded. Thus, in all probability H is the monosome in question.

Ovular transmission

Transmission of the monosomic condition through the egg parent was another characteristic used in identifying the monosomic type. A plant with 23 pairs of *Tabacum* chromosomes and a *glutinosa* monosome (symbol Hg) was pollinated by a normal plant (table 3). The differences between the tested and the check types were well within the limits of statistical error.

Cytogenetical analysis

While a comparison of the Purpurea monosomics gave a good indication as to the chromosome involved in the substitution, the analysis of certain crosses provided still better evidence. The cross (haplo-H Purpurea)×Holmes Samsoun gave monosomic offspring with 23 pairs of chromosomes and one univalent in the first meiotic metaphase (fig. 1). A few testcrosses were made between other monosomics and Holmes Samsoun which gave monosomic segregants with 22 pairs and three univalents (fig. 2, 3). This again confirmed the

evidence from morphology, pollen abortion and ovule transmission that H was the replaced chromosome. In the first cross both parents contributed 23 *Tabacum* chromosomes, all but the H. In addition, a single *glutinosa* chromosome was introduced by the pollen. In the other crosses the *glutinosa* chromo-

TABLE 3

Comparison of ovule transmission of monosomic condition.

POPULATION	TOTAL	MONOSOMIC	PERCENTAGE
Haplo-H×normal (CLAUSEN and CAMERON 1944)	888	625	70.4
Haplo-Hg×normal			
44 46*	19	16	
44 62*	43	34	
	62	50	80.6

* These were greenhouse winter and field summer populations from the same cross. The percentages were lumped.

Chi square = 2.95 P > 5%

some did not pair with the maternally derived H-chromosome, and these two univalents appeared in metaphase. The third univalent was the paternally derived chromosome for which the female parents were monosomic. The figures show clearly the difference between these monosomes and the members of the non-conjugating pair.

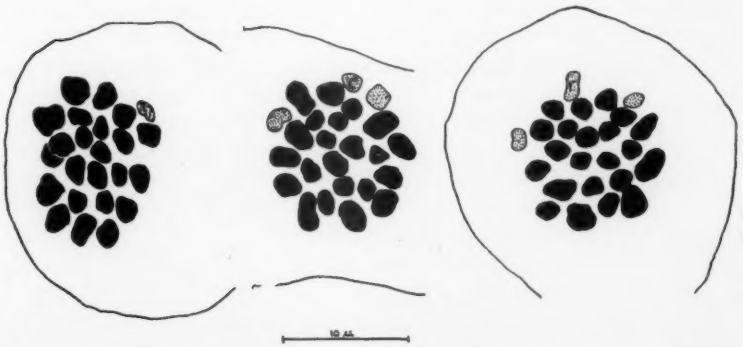

FIGURE 1 (left). Haplo-H×Holmes Samsoun, 23II+1I.—FIGURE 2 (center). Haplo-C ×Holmes Samsoun, 22II+3I.—FIGURE 3 (right). Haplo-F×Holmes Samsoun, 22II+3I.—The figures are from camera lucida drawings of microsporocytes at MI. Bivalents, solid black; univalents, stippled.

The monosomic sibs of the progeny from the cross (haplo-H Purpurea) ×Holmes Samsoun, selected on the basis of morphology, had in meiotic metaphase 23 pairs and one single chromosome, as noted above. These were pre-

sumably 23 *Tabacum* pairs and a *glutinosa* univalent, replacing the H chromosome of *Tabacum*. The results from a cross between Purpurea and such a plant (haplo-Hg) proved this to be correct. All 20 plants in the progeny, upon inoculation of their leaves in a moist chamber (GERSTEL 1943), produced the localized lesions characteristic of *N. glutinosa* and carried in the chromosome in question. This test made use of the fact that because of certation pollen with 23 chromosomes was practically eliminated (CLAUSEN and CAMERON 1944). The absence of segregation indicated that the necrotic reaction was carried in the monosome without a homologue—namely, the substitute for H.

For further verification the reciprocal cross haplo-H×Purpurea was made.

TABLE 4

Reciprocal crosses with haplo-Hg.

a) Purpurea×haplo-Hg

POPULATION	NECROTIC	MOTTLING	TOTAL
42 C	20	0	20

b) Haplo-Hg×Purpurea

POPULATION	NECROTIC DISOMIC	MOTTLING MONOSOMIC	NECROTIC MONOSOMIC	MOTTLING TERATOLOGICAL	TOTAL
44 46	2	16	0	1	19
44 62	8	33	1	1	43
Total	10	49	1	2	62

All the monosomic offspring were found to be susceptible to the mottling type of mosaic infection,—with one exception,—whereas the disomic plants produced necrotic lesions after inoculation. Again, the *glutinosa* monosome (Hg) carrying the necrotic factor was introduced by the eggs into the disomic segregants, but not into the monosomics. The one exceptional plant was probably due to a rare functional n−1 male gamete mating with an egg having 24 chromosomes, one of them Hg. These crosses and their results are summarized in table 4.

DISCUSSION

The peculiar fact that a *glutinosa* pair of chromosomes can replace a *Tabacum* pair without impairing development of a typical *Tabacum* plant has been previously discussed (GERSTEL 1943). The present data indicate that the *glutinosa* chromosome can take the place of the H chromosome of *N. Tabacum* not only as a single in spores and as a pair in the zygote, as was shown before, but also as the monosome in a monosomic. The single *glutinosa* chromosome must therefore contain all the genic material present in the H chromosome that is essential for the functioning of *N. Tabacum*. Such vital material must be contained in the H chromosome, because microspores with 23 chromosomes are rarely functional, and nullosomic plants cannot be obtained in *N. Tabacum*. How-

ever, the functional homology between H and Hg chromosomes is astonishing in view of the fact that the two species *N. Tabacum* and *N. glutinosa* are not thought of as close relatives.

A comparison with the compensating types of SEARS (1944) seems indicated. SEARS found in wheat that an extra pair of chromosome II compensates for the absence of pair XX, that is the combination tetra-II, nulli-XX is nearly normal, while tetra-II and nulli-XX are markedly abnormal. Similarly in tobacco a *glutinosa* pair compensates for the absence of the H pair. The physiological mechanism of the compensation in the two cases need not necessarily be similar.

There are probably some genic differences between the *glutinosa* chromosome and the *Tabacum*-H. The Purpurea material with the substituted chromosome was not suitable for comparison, since even the later generations were somewhat heterozygous, with many factors from Holmes Samsoun still present in the derivatives from F_1, other than those carried in the *glutinosa* chromosome. However, there is some other evidence available. VALLEAU (1942) and CLAYTON (communication, unpublished) have testified that they were unable to free strains of tobacco with the *glutinosa*-derived mosaic resistance from some undesirable features as slow growth, low yield, reduced leaf size and other quantitative traits. These were obviously conditioned by the *glutinosa* chromosome, being characteristic of that species.

Though they are homologous in function, the two chromosomes do not pair in the F_1 of the cross between Holmes Samsoun and Purpurea or any other tobacco variety tested (MALLAH 1943). It was suggested (GERSTEL 1943) that this lack of pairing may be due to structural differences between these two chromosomes. The material, unfortunately, is very little suited for a cytological confirmation of this hypothesis along the lines of the studies of inversion races of *Drosophila pseudoobscura* (DOBZHANSKY and EPLING 1944) or even of Lilium (RICHARDSON 1936) or Paeonia (STEBBINS 1938).

An asynaptic gene, acting on the hybrid background, may be mentioned as another possible cause of non-conjunction between the *glutinosa* and the *Tabacum* chromosomes, though the described cases of genic asynapsis affect all the chromosomes and not merely one pair (LEVAN 1940).

SUMMARY

It had been shown previously that mosaic resistance was introduced into Holmes Samsoun tobacco from *N. glutinosa* by substituting a pair of *glutinosa* chromosomes for one of *N. Tabacum*. By morphological and cytogenetical analysis, using the monosomic series of *N. Tabacum*, the replaced chromosome was identified as the H chromosome.

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LITERATURE CITED

BLEIER, H., 1933 Genetische und zytologische Untersuchungen von Weizenstämmen aus Weizen-Roggen-Bastardieruugen. Z. Pflanzenz. **18**: 191–211.

1934 Bastardkaryologie. Bibliogr. Genet. **11**: 393–489.

CLAUSEN, R. E., and D. R. CAMERON, 1944 Inheritance in *Nicotiana Tabacum*. XVIII. Monosomic analysis. Genetics **29**: 447–477.

DOBZHANSKY, TH., and C. EPLING, 1944 Contributions to the genetics, taxonomy, and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Instn. Washington Publ. **554**. 183 pp.

GERSTEL, D. U., 1943 Inheritance in *Nicotiana Tabacum*. XVII. Cytogenetical analysis of *glutinosa*-type resistance to mosaic disease. Genetics **28**: 533–536.

HOLMES, F. O., 1938 Inheritance of resistance to tobacco mosaic in tobacco. Phytopathology **28**: 553–561.

KATTERMANN, G., 1937 Das Verhalten des Chromosoms für Behaarung roggenbehaarter Nachkommen aus Weizenroggenbastardierung in neuen Kreuzungen mit Roggen und Weizen. Z. i.A.V. **74**: 1–16.

1938 Über konstante, halmbehaarte Stämme aus Weizenroggenbastardierung mit $2n=42$ Chromosomen. Z.i.A.V. **74**: 354–357.

LEDINGHAM, G. F., and W. P. THOMPSON, 1938 The cytogenetics of nomamphidiploid derivatives of wheat-rye hybrids. Cytologia **8**: 377–397.

LEVAN, A., 1940 The cytology of *Allium amplectans* and the occurrence in nature of its asynapsis. Hereditas **26**: 353–394.

MALLAH, G. S., 1943 Inheritance in *Nicotiana Tabacum*. XVI. Structural differences among the chromosomes of a selected group of varieties. Genetics **28**: 525–532.

NISHIYAMA, I., 1939 Cytogenetical studies in Avena. III. Experimentally produced eu- and hyperhexaploid aberrants in oats. Cytologia **10**: 101–104.

O'MARA, J. G., 1940 Cytogenetic studies in Triticale. I. A method for determining the effects of individual Secale chromosomes on Triticum. Genetics **25**: 401–408.

RICHARDSON, M. M., 1936 Structural hybridity in *Lilium Martagon album*×*L. Hansonii*. J. Genet. **32**: 411–450.

SEARS, E. R., 1944 Cytogenetic studies with polyploid species of wheat II. Additional chromosome aberrations in *Triticum vulgare*. Genetics **29**: 232–246.

STEBBINS, G. L., 1938 Cytogenetic studies in Paeonia II. The cytology of the diploid species and hybrids. Genetics **23**: 83–110.

VALLEAU, W. D., 1942 Control of the common mosaic disease of tobacco by breeding. Phytopathology **32**: 1022–1025.

ISOLATING BETTER FOUNDATION INBREDS FOR USE IN CORN HYBRIDS[1]

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THE problem of isolating better foundation corn inbreds—that is, new inbreds from open-pollinated varieties—is important. It is particularly important in the South. Inbreds for crossing and further selection are not as available as in the Corn Belt. Furthermore, there is a very strong tendency for the better inbreds selected from hybrid combinations to be earlier maturing than the mean of the parental lines. Consequently, there is less and less material to draw upon as later and later inbreds are required.

There have been marked improvements in the techniques of corn breeding, so that much more can be accomplished with a given effort. Accordingly, the number of reasonably good inbreds from a given sized program should be larger, with greater chances that a few will be superior. I wish in no way to belittle the importance of these advances. I do wish to consider whether some of the proposed methods are really superior and whether other methods may not offer a more promising basis for isolating better foundation inbreds. The problem will be considered in the light of available pertinent data and of certain genetic theories.

Under the characteristic system of selecting inbred lines as practiced in the 1920's, a number of once-selfed ears gave origin to an equal number of families. Several progeny lines of each family were grown each year, only the better ones being continued in the succeeding generation. No consideration was given to combining value until fixation had been partly achieved in S_3 or S_4. The families then were tested in hybrids and the better ones gradually brought into utilization. The number of families differed with the program. It will be convenient to refer to this as the older method. It depended on large numbers, on chance, and on what then was only a hope that the better inbreds could produce the higher-yielding hybrids.

More recently, it has been proposed (JENKINS 1935; SPRAGUE 1939) that a test of the original selfs in crosses with a variety (that is, "top crosses") could be substituted for the test in S_3 or S_4, saving the labor of carrying families which otherwise would be discarded later. The savings would be devoted, partly at least, to added selection for ear and kernel characters, and the like. This may be called the substitute method. Its justification rests primarily on an interpretation of a comprehensive experiment reported by JENKINS (1935). Before re-examining his data, it is desirable to consider briefly some correlation studies as evidence of the effectiveness of selection in practice.

[1] Joint contribution from the DIVISION OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE, and the TENNESSEE AGRICULTURAL EXPERIMENT STATION. Presented in part at a meeting of the AMERICAN SOCIETY OF AGRONOMY in Cincinnati, Ohio, November 12, 1943.

EFFECTIVENESS OF SELECTION IN PRACTICE

Correlation studies largely with S_3 or S_4 material have consistently shown, with minor exceptions, that higher-yielding inbreds tend to produce the higher-yielding hybrids. They also have shown that characters associated with vigor and productiveness in the inbreds were correlated, in general, with the yield of the hybrids. Most of the coefficients reported are not importantly high. This has led to an opinion, frequently expressed, that inbred performance has little relation to combining value. Most of the correlations, however, have been for the mean yields of two parents with the yields of their individual hybrids. But JENKINS (1929) has reported correlations for the yields of individual inbreds with the mean yield of their several crossbred progeny. The significance of these seems largely to have been overlooked. This method is comparable with that used for measuring the worth of top crosses as an indicator of combining value, and coefficients for the two criteria with mean hybrid yields are shown in table 1. The two sets of coefficients are not strictly comparable for various reasons. Nevertheless the measured performance of inbreds, as such, in S_3 and S_4 appears to have been about as good a criterion of combining value as was top-cross performance. To the extent that the methods of selecting used have chosen the higher-yielding inbreds, then, selecting for combining value will have been partly achieved.

More direct evidence on the effectiveness of selection, as well as on the possible influence of chance changes in combining value during inbreeding is available from JENKINS' (1935) experiment, previously referred to.

TABLE 1

Coefficients of correlation for the yields of inbred parents or of top crosses with the mean yields of the single crosses.

(Data from different experiments)

HYBRIDS CORRELATED WITH	PREVIOUS GENERATIONS OF INBREEDING									
	S_3			S_4		S_6				
Inbred parents[a]	0.25	0.64	0.67	0.41[c]	0.45[c]	—	—	—	—	—
Top crosses[b]		.53		0.53[c]		0.63	0.65	0.86	0.90	0.90

[a] JENKINS 1929.

[b] JENKINS and BRUNSON 1932.

[c] Yields for the two variables determined in different seasons.

Selection in successive generations

The data comprise the yields of top crosses made following seven out of eight generations of selfing and selecting in 14 families of Iodent and 13 families of Lancaster Surecrop, as carried in the Iowa corn breeding program. The plans for this program, as outlined in 1922, provided that no families be discarded until they had been tested in hybrid combination, unless they became unduly difficult to perpetuate. Accordingly, the families (originating with individual open-pollinated ears) used were a reasonably random sample of the two varie-

ties. Top crosses were attempted for two sister lines of each family in each generation, one in the continuous line of descent (the selected line) and one that had been discarded (the discarded sib). Heat and drought in the season when the top-crossed seed was being produced caused poor pollination. As a result, seed of some crosses was entirely lacking, and that of others came from too few ears adequately to sample the tester variety. JENKINS (1935) emphasizes this latter condition as a probable reason for some of the fluctuations evident in the data. To minimize this influence, his analysis was based on averages of performance, either directly or through analyses of variance.

Seven of the Iodent families and five of the Lancaster families were represented in the yield trials by a selected line in each generation. JENKINS (1935, tables 4 and 5) gives the average yields of these families for each generation and each variety. The trends from S1 to S2 were upward in Iodent and downward in Lancaster, with large differences in these opposite directions. Disregarding these trends, for which no reason is apparent, the average yield of the seven Iodent families was essentially constant from S2 to S8. The average for Lancaster was very irregular with a negligible upward trend. Analysis of variance showed that variation due to family was very significantly larger than that due to interaction between family and generation. On the basis of these comparisons, JENKINS concluded that the families had acquired their individuality as parents of top crosses very early in the inbreeding process, and that selection had not been effective in improving combining value through the successive generations.

TABLE 2

Acre yields (bushels) of families grouped according to better and poorer fractions in periods 2 and 4. "Better" fractions were: for Iodent, four of seven; for Lancaster, three of five.

Computed from, JENKINS 1935; tables 4 and 5

BETTER IN 2 BETTER IN 4				BETTER IN 2 POORER IN 4				POORER IN 2 BETTER IN 4				POORER IN 2 POORER IN 4			
FAMILY	PERIODS			FAMILY	PERIODS			FAMILY	PERIODS			FAMILY	PERIODS		
	2	3	4		2	3	4		2	3	4		2	3	4
I252	92.9	82.1	88.8	I197	87.5	80.7	77.3	I238	83.9	84.1	85.3	I185	80.7	71.5	78.6
I182	85.8	97.9	97.7	I154	85.2	90.4	81.6	I234	82.9	90.4	89.4				
Av.	89.4	90.0	93.3	Av.	86.4	85.6	79.5	Av.	83.4	87.3	87.4	Av.	80.7	71.5	78.6
L293	68.4	73.3	73.7	L317	77.4	75.9	69.6	L287	58.6	69.2	77.7	L309	56.9	63.6	64.9
L311	62.4	66.5	71.7												
Av.	65.4	69.9	72.7	Av.	77.4	75.9	69.6	Av.	58.6	69.2	77.7	Av.	56.9	63.6	64.9
G. Av.	77.4	80.0	83.0	G. Av.	81.9	80.8	74.6	G. Av.	71.0	78.3	82.6	G. Av.	68.8	67.6	71.8

It did not seem too surprising that the average combining value of several random families did not show an increase. Selection might well have been effective in some, not in others. Any individuality would be largely lost in such averaging. On the other hand, averaging the yields for the individual families

by two-generation periods would smooth the data without masking trends during the progress of selfing and selecting. Accordingly, the means for S_2–S_3, S_4–S_5, and S6–S8 were computed and designated as for "periods" 2, 3, and 4, respectively, thus reserving period 1 for S_1.

The families then were ranked in accordance with their top-cross yields in period 2 and in period 4. The higher-yielding four of the seven Iodent families and three of the five Lancaster families were designated as the better fractions, the others as the poorer. The families were then classified into those which were (A) better in both periods 2 and 4, (B) better in period 2 but poorer in

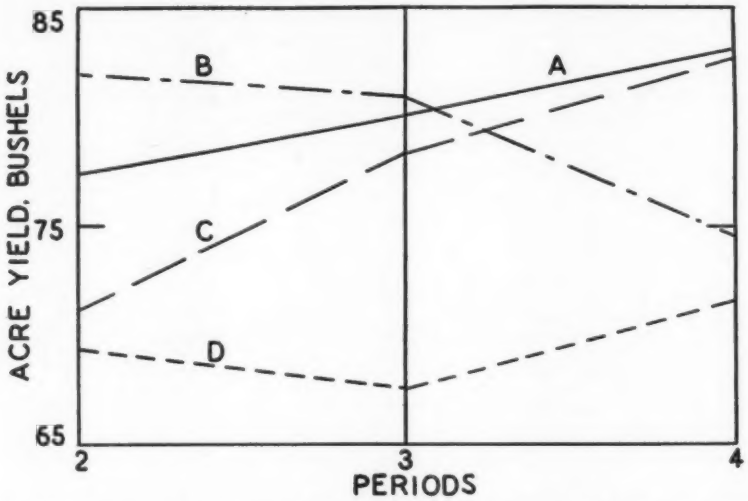

FIGURE 1.—The average acre yields in periods 2, 3, and 4 for top crosses of selfed lines of corn classed for crossbred performance in periods 2 and 4.

period 4, (C) poorer in 2 but better in 4, and (D) poorer in both periods. The yields by families and classes are given in table 2, and the averages for the classes are shown in figure 1.

The lack of correspondence between early and late performance is obvious. Discarding the five poorer families (C and D) in period 2 would have resulted in no larger a proportion of good families in period 4. On the other hand, the three families in C, having a very high yield in period 4, would have been eliminated. Early selection for top-cross performance in this material accordingly would not only have been a waste of time, but actually detrimental to the program.

It also seems obvious that the better families in period 4 were better, in part, because they had improved in combining value during the progress of selfing and selecting. This is further emphasized in figure 2, which shows the average yields by periods for the seven families that were better and the five that were

poorer in period 4. High performance at fixation appears to have originated with a trend in period 3 which was then maintained. In this connection, it should be emphasized that performance in period 3 was entirely independent of the classification system used.

Table 3 compares the top-cross yields of the selected and discarded sister lines in these 12 families. Differences in favor of the selected line are shown in the plus columns, and those in favor of the discarded sibs in the minus columns.

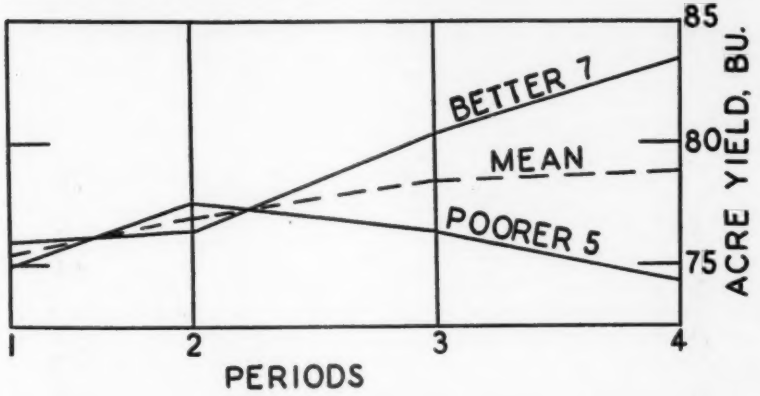

FIGURE 2.—The average acre yields in various periods for the top crosses of seven selfed lines of corn better in period 4, and of five lines poorer in that period.

TABLE 3

Differences in favor of the selected progeny (+) and of the discarded progeny (−) in families represented by selected progenies in all genrations.

(Compiled from tables 1, 2, JENKINS 1935.)

FAMILY	ACRE YIELDS IN FAVOR OF THE SELECTED PROGENY (+) AND OF THE DISCARDED PROGENY (−) IN THE SELFED GENERATIONS STATED											
	S1		S2		S3		S4		S5		S6	
	+	−	+	−	+	−	+	−	+	−	+	−
I182		9.3	7.0		13.6		4.7		6.2		7.7	
I234		8.9		3.4		12.6	1.4		4.6			4.2
I252	23.6		5.5		7.9		1.7			15.3		.9
I238	—	—	.7		1.8		3.2		3.5		5.6	
L287	—	—		7.0		10.6	17.7		6.8			5.3
L293	11.5		17.4		—	—	10.7		—	—		11.6
L311	—	—		16.0	—	—	7.9		—	—	—	—
Frequencies better 7	2	2	4	3	3	2	7	0	4	1	2	4
I154	5.9		0.1		9.9			5.7	—	—	5.1	
I185		15.7		0.7		0.3		2.4		6.2	6.1	
I197	6.9		20.9			.5		2.2	8.8			3.1
L317		5.6	1.8		20.7			9.9		6.2	—	—
L309	16.0			18.6		14.9		5.3	3.8		18.7	
Frequencies poorer 5	3	2	3	2	2	3	0	5	2	2	3	1

The total frequencies in the two directions are shown by generations for the groups of seven and five families which were better and poorer in period 4 The totals do not differ markedly until S4. Then, the selected lines were universally superior to their discarded sibs in the seven better families, and universally poorer in the five poorer families. A less marked, but similar situation occurred in S5. It was in the mean yields for these two generations that the trends shown in figure 2 originated.

As here analyzed, there appears to be little in the data to justify JENKINS' conclusion that the families acquired their individuality as parents of top crosses very early in the inbreeding process. On the contrary, there is every-evidence that the families altered their ranking for combining value. Further,

TABLE 4

Comparisons between selected and discarded sibling progenies in all families.
(Computed from JENKINS (1939), tables 1 and 2)

GENERATION[a]	TOTAL NUMBER	SIGNIFICANT		SIGNIFICANTLY IN FAVOR OF SELECTED PROGENY		
		NUMBER	PERCENTAGE	NUMBER	AS PERCENTAGE OF:	
					TOTAL	SIGNIFICANT
1[b]	22[b]	12[b]	55	10[b]	45	83
2	18	6	33	4	22	67
3	17	5	30	2	12	40
4	21	3	14	3	14	100
5	15	4	27	3	20	75
6	14	2	14	2	14	100
Total	85	20	24	14	17	70

[a] *Among* ears selfed the number of times stated.
[b] Not included in totals.

there is at least a strong suggestion that some of the changes were associated with successful and unsuccessful choices among S4 and S5 lines. The successful choices may well have been fortuitous. The important thing is that there was opportunity for selection and that the successful choices appear to have originated trends towards superiority. A more objective basis for selection at this stage accordingly should have some possibility of success.

Additional evidence as to the possibility of visual selection and its partial success in practice is available from the same experiment. Including all families and all generations, there were 107 comparisons between selected lines and their discarded sibs. The numbers and percentages of these comparisons that were statistically significant and that were significantly in favor of the selected lines are shown in table 4. Disregarding comparisons among S1 ears (from S0 plants), 70 per cent of the significant differences were in favor of the selected lines. Moreover, though there was a tendency toward fewer significant differ-

ences in S4 and later, a larger percentage of those which did occur were in favor of the selected lines. It was these comparisons which led JENKINS to conclude that selection had been generally effective in choosing the better siblings. This selection of course had been only visual, being based on selfed appearance in the progeny rows.

Combining value of lines within families

Experiments on the variation in combining value among sister lines within families afford another approach to the problem. Thus, JENKINS (1940) reported the yields for top crosses of sibling plants within S1 families. The variation was so low that only one plant in 40 would be expected to exceed the mean for the family by twice its standard error. This was noted as less than is statistically significant in the average run of corn experiments with five or six replications. SPRAGUE and BRYAN (1941) studied yields of top crosses of individual F_3 families and their F_4 progenies, from a cross between two long-time inbreds. They note that their results were in accord with those of JENKINS (1940). For reasons to be shown later, it seems at least possible that this indicated lack of variation among siblings is evidence of the inadequacy of top crosses to demonstrate it, rather than evidence of a real lack of variation. Certainly there is segregation for genes influencing the growth of the inbreds themselves during the early generations of selfing. In view of the correlations between inbred and hybrid performance already noted, it seems highly probable that these genes also influence combining value. (The influence that the characteristics of inbreds may have on their chances of elimination or selection in the earlier stages of a breeding program has been depicted in verse by RICHEY 1944.)

SELECTION AND GENES FOR YIELD

I wish now to consider selection from the standpoint of genes determining yield in corn. For simplification, this will be restricted to expectations under a specific working hypothesis. This hypothesis is not advanced as being a complete analysis of the genetic basis for yield in corn. It appears, however, to afford a reasonable approximation to the more important conditions dominating the effectiveness of selecting corn inbreds.

The hypothesis

The hypothesis assumes:

(1) Quantitative characters in corn are determined by a large number of genes having small, more or less equal effects and with dominance lacking (EMERSON and EAST 1913).

(2) Genes determining vigor in corn are in the dominant-recessive category, with the dominants more favorable for growth than their recessive alleles. This is no more than the generally accepted explanation of hybrid vigor (BRUCE 1910; KEEBLE and PELLEW 1910; RICHEY and SPRAGUE 1931). To the extent that these genes determine growth under a given environment, they also determine the ultimate expression of the quantitative characters and, consequently, yield.

(3) The effects of individual recessive genes are not equal. For convenience, however, genes of approximately equal effects may be discussed as in a group or order. Thus, at one extreme, are genes of the highest order, such as lethals or near-lethals. At the other extreme, are recessives of lowest-order effect, only slightly less favorable than their dominant alleles. Between these are genes of intermediate effects or orders.

(4) The frequency of particular recessives in open-pollinated corn will be inversely related to their deleteriousness. These frequencies will be determined by their mutation rates, the selection pressures against them (in favor of their more favorable dominant alleles), and by other factors (WRIGHT 1942, and earlier). Thus, recessives of highest-order effects will occur in very low frequencies because of the tremendous selection pressure against them. On the other hand, recessives only slightly less favorable than their dominant alleles will approach an equal gametic frequency of 0.5. Facts are lacking to warrant an assumption as to the particular nature of this inverse relation. For present purposes it will serve to take it simply as a reasonably high negative correlation.

(5) Linkage will impose a severe hindrance to independent recombination (JONES 1917; ANDERSON 1939). It will be ignored here, however, beyond this recognition of its important influence.

The problem then will be treated as being to isolate inbreds carrying the largest complements of favorable genes. The correlations between performance of inbreds, *per se*, and in crosses point to such inbreds as the most probable source for parents of high-yielding hybrids. Furthermore, it is taken for granted that the foundation inbreds to be isolated will at some time be a basis for further improvement. At such time, the inbreds carrying the most favorable genes would provide the most promising material. The discussion will be centered primarily on the action of genes in the dominant-recessive category, with only occasional reference to those lacking dominance. The latter also are important. They will differ from the other group, however, only in phenotypic expression which will be more direct, making them more amenable to selection.

Segregation, fixation, and time of selection

It is probable that there will be more different recessives of the lower orders than of the higher orders in the population. Even with equal numbers in the population, however, individual plants will carry more recessives of the lower orders because of their higher individual frequencies. The plants of a sample such as might be selfed to originate new inbred families, then, will tend to carry few recessives of highest-order effect, and these will differ from family to family. With genes of successively lower-order effects, the families will carry more and will have more in common. It is evident that the terms "highest-order effect" and "lower-order effect" are relative. They apply only to the particular sample being considered, whether variety, family, or group of lines.

Any method of selecting among the one-time selfs which would tend to retain the more favorable dominants and reject the recessives would raise the level of the selected material. The selected selfs, however, would be heterozy-

gous for different numbers of genes; relatively few of the higher orders and more of the lower orders, as noted. For the moment, it is desirable simply to explore the relation of random segregation and fixation to the time of opportunity for selection in a family under continued self fertilization. This may be done conveniently by examining segregation at five loci of equal order of effect.

Under continuous selfing, a line heterozygous for five dominant-recessive genes will be distributed phenotypically according to the formula:

$$\left[\frac{(2^r+1)\mathrm{D}-+(2^r-1)\mathrm{dd}}{2(2^r)}\right]^5$$

with $(\mathrm{D}-)^5$ indicating phenotypes with five dominant genes (symbolized by D), with $(\mathrm{D}-)^3\mathrm{dd}^2$ indicating phenotypes with three dominants and two recessives, etc.

As r (the segregating generation) increases with inbreeding, (2^r+1) and (2^r-1) approach equality, and, without selection, the population approaches a distribution for dominant and recessive homozygotes described by:

$$\left[\frac{1\ \mathrm{DD}+1\ \mathrm{dd}}{2}\right]^5$$

The variance within the individual lines of descent halves each generation, and the opportunity for selecting within such lines decreases as outlined by JENKINS (1940). On the other hand, the variance within the population as a whole increases. Selection among the progenies of initial segregates accordingly is more certain than was selection among the segregates themselves.

These statements are based on the assumption that all classes are propagated equally. Actually, of course, only exceedingly small samples of the populations can be grown. If random, however, they will tend to derive from the modal classes of the distribution. In the early generations, these classes will comprise individuals which are highly heterozygous. In later generations of inbreeding without selection, they would comprise individuals having homozygous favorable and unfavorable genes in approximate equality. The situation is illustrated in the upper half of figure 3, which shows graphically the percentage phenotypic distribution in F_2 and in F_3 and F_4 under random propagation—that is, with no, or non-effective selection. The dominant homozygotes are shown by the self-black areas. Potentially favorable genotypes are frequent in F_2, but are largely heterozygous. By F_4, the relative frequencies in the favorable classes have decreased materially, although favorable individuals are much more homozygous when they do occur. It is obvious, then, that the chances are very poor for obtaining a reasonably fixed, highly superior genotype in any single generation. In the earlier generations, superior individuals will be largely heterozygous, and in the later generations they will occur with negligible frequencies.

But it is unnecessary to confine selection to a single generation, and continued selection in successive generations offers definite advantages. Thus, assume a selection criterion which can distinguish effectively the action of two

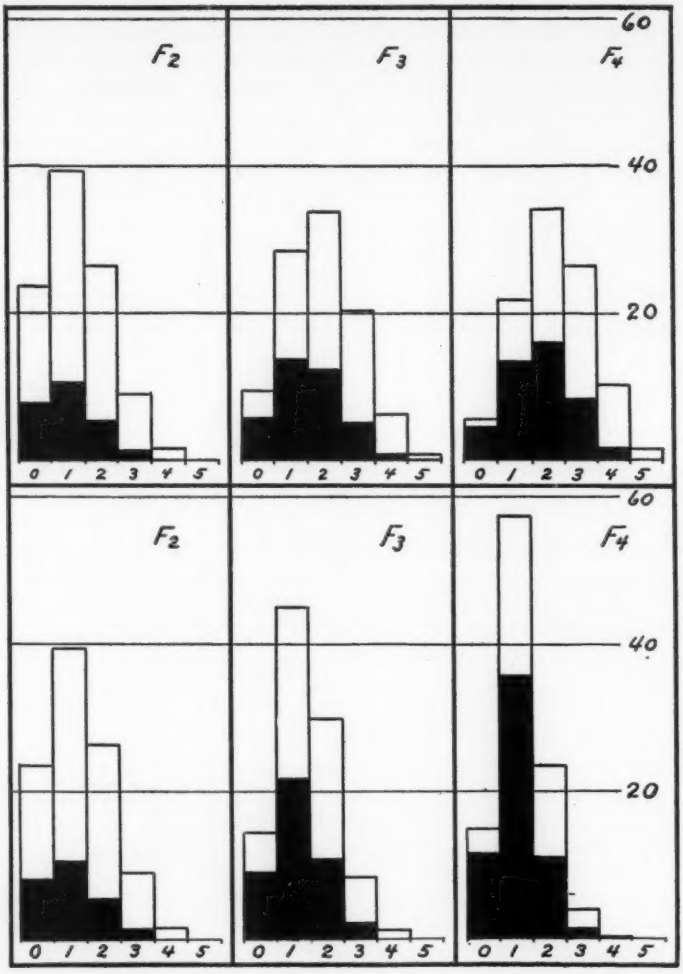

FIGURE 3.—The percentage distributions for plants from an F1 heterozygous for five pairs of dominant-recessive alleles;
Upper half, all classes propagated equally.
Lower half, Only phenotypes having 0 or 1 expressed recessive propagated.

or more of the five recessive genes, and that all classes having fewer than that number of expressed recessives are propagated equally, the less desirable classes being discarded. The actual frequencies in the two favorable classes would be the same as under random propagation of all classes, whereas the frequencies in

the other classes would become less. The percentage frequencies of the favorable classes accordingly would increase. The situation is illustrated in the lower half of figure 3. This shows the distribution for the same F_2 and those for the F_3 and F_4 populations derived from it by propagating only those classes having zero or one expressed recessive. The marked increase in the proportions of the selected, more favorable classes, in contrast to the increase in the less favorable classes under random propagation (upper half of figure 3), is eloquent evidence of the value of effective selection in F_2 and F_3. Moreover, the increase in homozygosis within the selected classes will have been the same as it would have been under random propagation.

The principles illustrated in figure 3 apply generally, regardless of the number of genes involved. Continuous effective selection will establish a population at or above the limits of effectiveness. Random propagation or non-effective selection will result, on the average, in mediocrity. The importance of continuity of selection cannot be over-emphasized. The selection of an inferior genotype at any stage will immediately establish the upper limit for the family. The number of genes involved will, of course, influence the possibilities of effective selection. COLLINS (1921) showed the fallacy of expecting the extreme (favorable) classes even to occur in ordinary F_2 samples of an F_1 heterozygous at only 20 loci. That, however, does not preclude the possibility of some effectiveness, as shown by the more general choice of the superior sibs in the Iowa program (table 4).

Selection criteria

The problem of selecting for ultimate combining value under the hypothesis outlined can be approached conveniently by considering first the performance of the progeny of a single S_1 plant, heterozygous at three loci, when tested in different ways. The plant will be taken as heterozygous at *A* with a ten-unit effect, and at *B* and *C* with five-unit effects each. The F_2 plants from such a self would have the normal genotypic distribution for a triple heterozygote. Since *b* and *c* are equal in effect, there would result the 18 phenotypes shown in table 5.

The "Yield units above base for recessive *a b c*" are for the F_2 plants and for progenies derived from them in various ways. Thus, the second column shows the plus values for F_3 progenies from the F_2 plants of the different classes. The remaining columns show the values for crosses of the F_2 plants by different testers. The third yield column assumes a backcross to the F_1 in which *ABC* and *abc* would be equally represented. The fourth column shows the values for crosses to a tester variety which, by assumption, has two recessive *b c* alleles for each recessive *a*, or a perfect inverse relation between effect and frequency. In the tester assumed for obtaining the fifth column, the relation between effect and frequency is intermediate.

Except in degree, the crosses by different testers all tell the same story. In contrast to those of the selfed progenies, the narrow ranges and low variances for the crosses show clearly the tendency of test crosses to mask the inherent variation. In later generations, under continued selfing, the variances would all

increase. The ranges would remain constant, however, and the variation among the selfed progenies would continue to be much greater than that among the crosses, as shown by the variances at homozygosis at the bottom of table 5.

Crossed performance indicates the multi-heterozygous class (Class 6) as having a value equal only to that of the mean. It is this mean that JENKINS

TABLE 5

Mean yield units above base for F2 plants of different genotypes and for progenies derived from them in various ways.

CLASS	F[a]	F2 GENOTYPES	Yield units above base for *abc*				
			FOR PROGENIES DERIVED FROM F2 BY:				
			F2 PLANT	SELFING	CROSSING BY:		
					.5 *ABC* .5*abc*	.9 *A*+.1 *a* .8*BC*+.2*bc*	.75*A*+.25*a* .6*BC*+.4*bc*
1	1	*AABBCC*	20	20.00	20.00	20.00	20.00
2	4	*AABBCc* and *AABbCC*	20	18.75	18.75	19.50	19.00
3	2	*AaBBCC*	20	17.50	17.50	19.50	18.75
4	4	*AABbCc*	20	17.50	17.50	19.00	18.00
5	8	*AaBBCc* and *AaBbCC*	20	16.25	16.25	19.00	17.75
6	8	*AaBbCc*	20	15.00	15.00	18.50	16.75
7	2	*AABBcc* and *AAbbCC*	15	15.00	17.50	19.00	18.00
8	4	*AABbcc* and *AAbbCc*	15	13.75	16.25	18.50	17.00
9	4	*AaBBcc* and *AabbCC*	15	12.50	15.00	18.50	16.75
10	8	*AaBbcc* and *AabbCc*	15	11.25	13.75	18.00	15.75
11	1	*AAbbcc*	10	10.00	15.00	18.00	16.00
12	1	*aaBBCC*	10	10.00	15.00	19.00	17.50
13	4	*aaBBCc* and *aaBbCC*	10	8.75	13.75	18.50	16.50
14	2	*Aabbcc*	10	7.50	12.50	17.50	14.75
15	4	*aaBbCc*	10	7.50	12.50	18.00	15.50
16	2	*aaBBcc* and *aabbCC*	5	5.00	12.50	18.00	15.50
17	4	*aaBbcc* and *aabbCc*	5	3.75	11.25	17.50	14.50
18	1	*aabbcc*	0	.00	10.00	17.00	13.50
		Mean[b]	15.	12.50	15.00	18.50	16.75
		Variance[b]	28.1	21.1	4.7	.4	1.8
		Variance[c]	37.5	37.5	9.4	.8	3.6

[a] Frequency.
[b] Weighted for frequency, as tabled.
[c] Weighted for frequency, at homozygosis.

(1940) found was so seldom exceeded significantly (one in 40) by the test crosses of F2 siblings. But this class has the possibility of producing a homozygous dominant with maximum combining value. Selfed progeny performance indicates Class 6 as superior to the mean and therefore more nearly estimates its potential breeding value.

The crosses systematically fail to distinguish between the effect of two recessives of equal importance when they are at one or at two loci (Classes 4 and 7). Class 7 is homozygous recessive at one locus and cannot be improved; Class 4 is a double heterozygote from which there is the possibility of isolating a homozygous dominant. It accordingly is the potentially superior breeding material which the selfed-progeny performance indicates it to be. This systematic tendency, in fact, does not obtain with the selfs. Here, too, potentially superior genotypes occasionally may be indicated as equal or inferior to poorer ones, as in Classes 6 and 7, or 11 and 12. This, however, results from an accidental balancing of effects here and there; a condition which operates also among the crosses, in addition to the systematic influence noted. This weakness of crosses as testers is more important for genes of higher frequencies (with lower effects). It is at a maximum in F_2, when homozygotes and heterozygotes are equal in number, and reduces gradually to nil at homozygosis.

There is a marked difference between the influence of genes *A*, *B*, and *C* on the performance of the selfs and the crosses. The performance of the selfed progenies (column 2) is directly related to the combined number and importance of the genes in the parent plants. Thus, one dominant *A*, of ten-unit effect, is equivalent to combined *B C* with a total effect of ten units. Among the crosses as shown in column 4, on the other hand, the perfect inverse relation between effect and frequency results in *A* having an effect equal only to that of *B* or *C*. The effects in the last column are intermediate between those in columns 2 and 4. Selection on the basis of selfed performance accordingly would be primarily for *A*, the gene of major effect, and only secondarily for *B* and *C*, the genes of second-order effect. Among the crossed progenies, on the other hand, selection would be for *A*, *B*, and *C*, either equally or more nearly so. As genes of major effect were eliminated by selection, selfed and crossed performance would tell more and more nearly the same story.

The tendencies operating within a family of lines as illustrated in table 5, are general and will operate regardless of genetic complexity. They also will operate as between families, but their impact will differ. There will be much more variation among different S_1's than within their individual progenies. Thus, SPRAGUE (1939) and SPRAGUE and BRYAN (1941) have reported yield ranges for top crosses of S_1's entirely adequate to permit practical selection among them. The masking of variation by top crosses accordingly is less important among families, though practically a bar to the use of top crosses for selecting within families. Again, the lesser domination of cross performance by genes of major effect should make crosses more importantly superior in selecting among S_1's. But it must be emphasized that selection differs in function during the earlier and later stages of the program. At first, the purpose is to choose the material from which the better combining lines can be isolated. At fixation, it is necessary only to determine which are the better combining lines.

The values for the F_2 plants (first column) are perfect criteria of their potential breeding possibilities. Under the conditions assumed for the example, however, they are not as good a selection criterion as is selfed progeny performance, inasmuch as the latter indicates probabilities as well as possibilities. The

individual F_2 plants have the added disadvantage that they are subject to the influence of a single, highly localized environment. (This disadvantage can be overcome in a species that can be propagated vegetatively.) Even so, the F_2 plants may be superior to the selfed progeny performance when genes of major influence are involved. Thus, the progeny of an excellent plant heterozygous for simple, recessive barrenness would have a very low yield. It is obvious, however, that this would be a poor reason for discarding it. The barrenness would quickly be eliminated from the line with further selection.

THE PLACE OF DIFFERENT PROCEDURES

There appears little reason to question the existence of these tendencies, though lack of necessary data precludes any very definite conclusions as to their relative importance. The planning of a breeding program, however, requires that tentative decisions be made in accordance with one's best judgment of such facts as do exist. On this basis, the following seem logical evaluations for the different procedures.

The use of either selfed or crossed progeny performance as a basis for selecting among or within families in the initial stages of a breeding program is not warranted.

Selfed progeny performance will provide an increasingly accurate measure for selecting within families as the program advances. Crossed performance will become less useful for this purpose.

Both selfed and crossed progeny performance improve in value for selecting among families with the advance of the program, and will come ever nearer to telling the same story. Crossed performance can more easily be determined experimentally. It also should remain somewhat superior to selfed performance, though how much is not clear. For both reasons, crossed performance will provide a more satisfactory practical criterion for selecting among families as early in the program as either criterion can profitably be used.

The stage at which crossed performance will become a practical criterion of breeding value cannot be defined absolutely. It should distinguish safely among many families after three generations of selfing, among more families after four generations, and among most families after five. The best stage to initiate its use, then, will depend upon the relative importance of time and certainty in the particular program. A happy balance might be after four generations.

Choice of the best individual plants will select for the total of expressed dominants, whether homozygous or heterozygous, which provides a good measure of breeding possibilities. The disturbing influence of a very localized environment limits its effectiveness. Until progeny selection becomes practically useful, however, it is the only alternative as regards genes in the dominant-recessive category.

Progeny performance provides a sound basis in visual selection for characters controlled by genes lacking dominance. Some of these characters, as ear size and number, are very directly concerned with yield. Others, such as erectness of plant and husk protection, determine general desirability. Resistance

to some diseases involves dominance; others not. A judicious combination of progeny selection for "type," and individual plant selection for vigor should be superior to either basis alone.

These conclusions lead essentially to the practices followed in the older methods. Those methods need no defense. They boast their success from a million signs along the highways of the Corn Belt. They have been improved in detail. They should be improved further as facts permit. But they should not be discarded for a newer method until it has been reasonably proven sound. No evidence has been found for any important advantage in testing for combining value in S_1 or S_2 instead of S_3 or S_4. On the other hand, early elimination on the basis of tested combining value can be detrimental. Nor has any other system of direct selection been found that promises importantly greater effectiveness than the older methods. This is not defeatism, but realism. It suggests the use of a different approach.

CUMULATIVE SELECTION

The approach I wish to suggest is: First, selection as outlined; second, single crossing among the high combiners; and third, further selection. There is only one new thing in this approach—namely, that the three elements be recognized at the beginning of the program as definite steps in an integrated system for selecting new inbreds from an open-pollinated variety. To emphasize this philosophy, the system as a whole is being designated "Cumulative Selection."

The possibilities and methods of selecting in the initial stage have been discussed. The families for crossing are to be chosen directly on the basis of their performance in the tester crosses. Crosses with varieties (JENKINS and BRUNSON 1932) or with single crosses (BEARD 1940) as testers could be used. But it is important that the high combiners be determined with considerable certainty. This indicates testing not before four, and probably not before five generations of selfing. Or, a preliminary crossing test after three, with a second after five generations would seem practical. The families chosen after the first test could be tried in various combinations for immediate utilization; those chosen after the second test would be used for crossing and further selection.

Under this thesis, selection during the first cycle will have eliminated gross recessives. It also will have been reasonably successful in fixing the dominants in the next lower order of effect. Consequently, selection during the second cycle can well be on a basis of selfed progeny performance. That this can be done simply is shown by experiments reported by HAYES and JOHNSON (1939). They found that the total yield of second-cycle inbreds from two nursery rows, including hand-pollinated and open-pollinated plants, provided a reasonable criterion of their combining value. Their results are encouraging also as to what may be expected from the second cycle of selection. Using selections from crosses between previously established inbreds, they found that those from crosses of two high-combining parents tended to be high combining; also that a considerable number of high combining lines could be derived from crosses in which only one of the parents had high combining value.

Finally, it is emphasized that the conclusions reached and the procedures

recommended are tentative. As such, they are subject to revision if and when additional facts indicate they are unsound in any detail. As of the present, however, the facts and theories considered in reaching these conclusions seem to be in agreement with each other and with the general experience of corn breeding; also they point to "Cumulative Selection" as currently offering the most promising approach to the isolation of better foundation corn inbreds. Whether "better" may mean only a higher mode for combining value is not known. It seems reasonable, however, at least to hope that a higher mode would carry with it some higher extremes. If so, some families resulting from cumulative selection should be superior to the best derived by straight selection. If not, there should be a larger number of usable families from a given sized program.

SUMMARY

It was shown that the yields of S_3 and S_4 inbreds are better criteria of their value in hybrids than generally has been recognized.

A re-analysis of JENKINS' (1935) data indicates that changes in the rank of 12 families for combining value from S_2–S_3 to S6–S8 were associated with successful selections among S_4 progenies. Furthermore, that discarding the poorer families on the basis of top-cross tests in S_2 and S_3 would have eliminated three families whose average combining value in S6–S8 practically equalled that of the best families which would have been retained.

A working hypothesis is advanced as to the operation of genes determining yield in corn. The essentials of this hypothesis are that genes determining quantitative characters lack dominance; that genes in the dominant-recessive category are concerned with vigor; that individual genes differ in effect; and that the frequency of a recessive in a population will be in inverse relation to its deleteriousness. The expectations under this hypothesis are explored, with the following conclusions.

Test crosses are a good criterion of combining value at any stage in the program, as of that time. They are not good indicators of prospective combining value, however, until fixation has reasonably been achieved.

Selfed performance on a progeny basis is not a good criterion of ultimate combining value until selection has eliminated recessives of larger individual effects and lower frequencies.

With effective selection against recessives of major effect and progress toward fixation, progeny performance of selfs and of crosses will tend to tell more nearly the same story. Selfed performance then will be satisfactory for selecting within families, while crossed performance will be more suitable for selecting among families.

A program based on these principles is outlined and designated as Cumulative Selection. This is a single, integrated system, comprising: (1) an initial period of visual selection for individual plants in desirable progenies; (2) testing for combining value; (3) crossing among the high combining families; and (4) a second cycle of selection based on selfed progeny performance.

LITERATURE CITED

ANDERSON, E., 1939 The hindrance to gene combination imposed by linkage: an estimate of its total magnitude. Amer. Nat. **73**: 185–188.

BEARD, D. F., 1940 Relative values of single crosses and an open-pollinated variety as testers of inbred lines of corn. Abstracts of Doctoral Dissertations (Ohio State Univ.) **33**: 9–18.

BRUCE, A. B., 1910 The Mendelian theory of heredity and the augmentation of vigor. Science **32**: 627–628.

COLLINS, G. N., 1921 Dominance and the vigor of first generation hybrids. Amer. Nat. **55**: 116–133.

EMERSON, R. A., and E. M. EAST, 1913 The inheritance of quantitative characters in maize. Nebr. Agric. Expt. Sta. Res. Bull. **2**: 120 pp.

HAYES, H. K., and I. J. JOHNSON, 1939 The breeding of improved lines of corn. J. Amer. Soc. Agron. **31**: 710–724.

JENKINS, M. T., 1929 Correlation studies with inbred and crossbred strains of maize. J. Agric. Res. **39**: 677–721.

1935 The effect of inbreeding and of selection within inbred lines of maize upon hybrids made after successive generations of selfing. Iowa State Col. J. Sci. **9**: 429–450.

1940 The segregation of genes affecting yield of grain in maize. J. Amer. Soc. Agron. **32**: 55–63.

JENKINS, M. T., and A. M. BRUNSON, 1932 Methods of testing inbred lines of maize in crossbred combinations. J. Amer. Soc. Agron. **24**: 523–530.

JONES, D. F., 1917 Dominance of linked factors as a means of accounting for heterosis. Genetics **2**: 466–479.

KEEBLE, F., and C. PELLEW, 1910 The mode of inheritance of stature and of time of flowering in peas (*Pisum sativum*). J. Genet. **1**: 47–56.

RICHEY, F. D., 1944 The shattered dram of a corn breeder. J. Amer. Soc. Agron. **3**: 267.

RICHEY, F. D., and G. F. SPRAGUE, 1931 Experiments on hybrid vigor and convergent improvement in corn. Tech. Bull. U. S. Dept. Agric. **267**: 22 pp.

SPRAGUE, G. F., 1939 An estimation of the number of top-crossed plants required for adequate representation of a corn variety. J. Amer. Soc. Agron. **31**: 11–16.

SPRAGUE, G. F., and A. A. BRYAN, 1941 The segregation of genes affecting yield prepotency, lodging and disease resistance in F_3 and F_4 lines of corn. J. Amer. Soc. Agron. **33**: 207–214.

WRIGHT, S., 1942 Statistical genetics and evolution. Bull. Amer. Math. Soc. **48**: 223–246.

NORMAL SEGREGATION WITHOUT CHIASMATA IN FEMALE DROSOPHILA MELANOGASTER

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FOR most chromosomes, segregation at meiosis depends upon an association or conjunction of homologs at metaphase of the first meiotic division. Generally the required state of metaphase conjunction is itself dependent upon an antecedent association or synapsis of homologous chromosomes during the inception of meiotic prophase. Following the onset of synapsis, homologous chromosomes may undergo reciprocal breakage and a new union of non-sister chromatids with consequent genetic crossing over and the formation of chiasmata. The hypothesis that the chiasmata observed by the cytologist are the direct consequences of such interchanges or crossing over was first put forth by JANSSENS (1909, 1919a, 1919b, 1924). WILSON and MORGAN's (1920) critical review of JANSSENS' hypothesis in effect drafted our present "chiasma-type theory," while DARLINGTON (1930, 1931a) supplied the first rigorous cytological proofs that crossing over gives rise to chiasmata and that every chiasma represents an exchange of partners between non-sister chromatids within bivalents and multivalents.

Following the events of crossing over, the chromosomes attain their condensed state by the completion of their meiotic coiling and the acquisition of accessory substances (or "matrix") and perhaps also a pellicle. While at metaphase the bivalent has its definitive form, yet the mechanism by which the homologous chromosomes are held together and thus have their coorientation on the spindle assured remains difficult to elucidate. It is conceivable that homologous chromosomes which have synapsed may remain associated until metaphase by a persistence of the "forces" which brought about the earlier synapsis, or by chiasmata, or by the development of partially-common matrices or pellicles, or by some mechanism associated with the coiling process, or by some new agent developed during prophase, or perhaps by a combination of two or more of these possible binding agents. Nevertheless, as is well known, cytologists have focused their attention almost exclusively on the possible role of the chiasma in guaranteeing conjunction and therefore segregation at meiotic metaphase, other factors having been for the most part overlooked or ignored.

The development by DARLINGTON (1931a, 1932) of a simple mechanical interpretation of the basic differences between meiosis and mitosis (the so-called "precocity hypothesis") led to the most vigorous advance and stimulating period of research that cytology has known since 1900. But one characteristic of that period of cytogenetic synthesis was a narrow preoccupation with only those structures, events, and assumptions which are directly involved in

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the hypotheses formulated by DARLINGTON and his disciples. By the rule of logical parsimony, of all the possible mechanisms of conjunction at meiotic metaphase only the chiasma is required by the propositions of the precocity hypothesis. Thus the "chiasma hypothesis of metaphase pairing" became a corollary of the precocity interpretation of meiosis. This corollary, it should be emphasized, seemed well substantiated for the case of large chromosomes by observations upon multivalent formation in polyploid liliaceous plants (DARLINGTON 1929, 1931a, 1931b and later). Where chromosomes proved too small or too poorly fixed for critical study, on the other hand, little hesitance or reserve has been shown by most authors in deducing *ex hypothesi* that chiasmata are providing the mechanism of metaphase conjunction. Although the precocity hypothesis is now known to be in manifest disagreement with the observations of a large number of independent workers, and is accordingly no longer a valid tool for further research (SAX 1936; HUSKINS 1937; COOPER 1938; BAUER 1939; HUGHES-SCHRADER 1940; SCHRADER 1944; and others), its ancillary concept which holds the chiasma to be the essential condition for segregation remains one of the most widely applied notions in cytogenetics. In spite of the fact that there is now a fairly impressive list of forms in which chiasmata are not necessary for segregation in at least one sex (see HUGHES-SCHRADER 1943a, 1943b; COOPER 1944), the general assumption of the DARLINGTON school now appears to be that chiasmata are universally essential for segregation in the meiosis of at least the opposite sex of these exceptional forms. Thus one sex is assumed to have developed a new or special mechanism of metaphase conjunction, whereas the other sex is held to retain the assumedly primitive method of conjunction by chiasmata. Because such a line of reasoning inexorably demands that crossing over underlies meiosis and sexual reproduction, it is not surprising to find that DARLINGTON (1939, p. 16) holds the view that "It is the central fact of genetics."

But are crossing over and the resultant chiasmata invariably necessary for orderly segregation in at least one sex of these forms? If not, the chiasma hypothesis of metaphase pairing certainly becomes suspect for all forms with small chromosomes, the precise details of which are difficult to ascertain, or whose chromosomes have been only casually investigated. The most promising organism for an investigation of this question seems to be *Drosophila melanogasier*. It is fairly definitely known that in this and in related species (*D. pseudoobscura*) crossing over and chiasmata either do not occur at all or are exceedingly rare in meiosis of the male (review and analysis in COOPER 1944). In the female a satisfactory cytological study of meiosis appears unfeasible at the moment, but the problem may be very elegantly attacked by purely genetic methods in this sex. On the basis of the chiasma hypothesis of metaphase pairing, approximately fifty percent of all non-crossover tetrads should give rise to non-disjunctional gametes with the consequent appearance of a proportional number of matroclinous female and patroclinous male exceptions among the offspring. GERSHENSON (1935), STONE and THOMAS (1935), and STURTEVANT and BEADLE (1936), studying segregation in X-chromosome inversion-heterozygotes, maintain that although in certain of their crosses there occurs

a very considerable percentage of noncrossover tetrads (hence chiasma-free bivalents), there is no corresponding increase in the matroclinous female exceptions above the normal rate. The observations of STURTEVANT and BEADLE alone would have disposed of the chiasma hypothesis as a reliable basis for general deduction were it not for the fact that the small right arm of the X (KAUFMANN 1934; PROKOFIEWA 1935) was not genetically marked in their experiments. It therefore remained possible that undetected exchanges or chiasmata in the right arm were actually guaranteeing normality of segregation in these females. The experiments described below show that this is not the case and that normal segregation of the sex chromosomes of female *Drosophila melanogaster* is independent of crossing over, as STURTEVANT and BEADLE, GERSHENSON, and STONE and THOMAS concluded.

METHODS

The experiments were all conducted at 25° ± 0.2°C. Single virgin females heterozygous for the two special X chromosomes described below were mated in shell vials with one (or rarely, two) males for a 24-hour period. The couples were then transferred without etherization to freshly yeasted half-pint bottles containing a non-agar, cream of wheat and molasses food prepared according to the directions of SPASSKY (1943). For the most part, the flies did very well on this medium, although occasional bottles turned soupy and yielded relatively small progenies. As soon as pupae appeared, the parents were removed from the bottle. Flies were counted daily from onset of eclosion of the adults until the end of the period of emergence.

Egg collections from single females and rearing of flies from the eggs were carried out in the following manner. Glass rings 18 mm in external diameter by 4 mm in depth were cut from soft glass tubing. Ordinary microscope slides were cut into rectangles 20 mm × 45 mm in size. An area sufficiently large to encompass the base of one of the glass rings was then painted with a melted mixture of vaseline and soft paraffin near the middle of the slide. A glass ring was pushed gently down on the warm wax so that a water-tight cylindrical cell about 4 mm deep was formed. Ordinary food blackened with washed animal charcoal was run into the cell in sufficient quantity to give a smoothly rounded mound of food rising above the rim of the glass ring. When hard, the surface of the food was painted with a thin yeast suspension, care being taken to keep the yeast from wetting the rim of the cell. The glass slip carrying the food was then placed in an 80 mm × 20 mm shell vial, and a previously mated female and male were transferred into it without etherization. Thereafter the vial was plugged with cotton, and during the egg laying period placed on its side, food uppermost, in the incubator.

Flies were transferred without etherization to vials containing fresh cells of food at the end of each 12- or 24-hour interval of egg laying for a period of six days, at the end of which time the egg collections were arbitrarily terminated. Eggs were counted by means of a dissecting microscope immediately following transfer of the parents by removing the slide from the vial. After the first

counting of the eggs, the slide was returned to the vial. A drop of distilled water was then run into the vial in such a way that it was held by capillarity along the lower borders of contact of the glass slip with the wall and bottom of the vial. By this means excellent humidity conditions were preserved during hatching of the eggs in spite of the fact that only a cotton plug was used. About 25 to 30 hours after the initial egg count, the numbers of inviable eggs were determined. Thereafter the glass ring with the contained food and larvae was slipped off the slide and placed in a freshly yeasted half-pint bottle of food. In this way there was virtually no handling of eggs or early larvae, a fact which may help account for the surprisingly high viability shown by the figures of table 2.

DESIGN AND RESULTS OF THE EXPERIMENT

Two special X chromosomes were made up in stock for these experiments (fig. 1). One of them contains both the delta-49 (*dl-49*) and B^{M1} inversions,

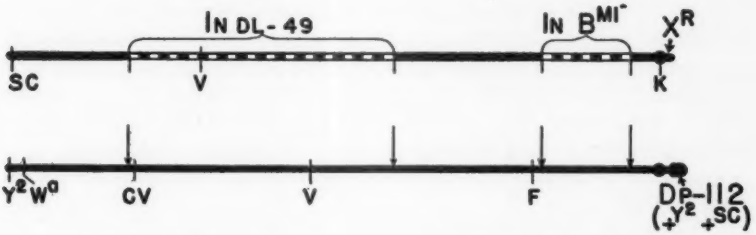

FIGURE 1. Maps of the X chromosomes used. The loci of the genes involved and the approximate limits of the inversions are as follows: y^2(0.0), *sc*(0.0+), *cv*(13.7), *v*(33.0), *f*(56.7), *Indl-49*(12± −41±), *In*B^{M1}(57± −67±). The actual sizes of X^R and *Dp112* are not known, but it may be taken for granted that they are very small. DOBZHANSKY (1932) states the cytological length of *Dp*(1;*f*)*112* to be equal to or smaller than the diameter of the fourth chromosome. The kinetochore is labelled k, and the vertical arrows pointing to sections of the *Dp112* chromosome indicate the approximate endpoints of the inversions in the other X.

while the other has a small duplication attached to the right arm (X^R) of the X. Both chromosomes carry the recessive mutant vermilion (*v*), so that when females carrying both these chromosomes are crossed to non-vermilion males all matroclinous female exceptions in the offspring are readily detected by their vermilion eyes. Crossing over between these two X's can be determined by the phenotypes of the regular male offspring Since the marking genes to the left of *dl-49* are different in the two chromosomes, crossovers in this region are readily detected, except for simple exchanges of scute (*sc*) for yellow^2 (y^2) into the chromosome bearing duplication 112 (*v. infra*). Crossveinless (*cv*) and forked (*f*) in the noninverted chromosome make it possible to detect exchanges between the two inversions. Inversion B^{M1} itself is associated with a weak Bar effect and so provides its own marker. Since y^2 at the left end of the chromosome bearing the duplication and *sc* at the left end of the inverted chromosome

are both covered by their wild type alleles in duplication 112 (*Dp112*), detection of exchanges to the right of inversion B^{M1} (that is, between B^{M1} and the kinetochore, or within X^R) is reliably accomplished.

As STURTEVANT and BEADLE (1936) have shown, single exchanges within inversions will not ordinarily be recovered, even though they may be expected to occur. Such single exchanges most probably result in a chromatid bridge and an akinetic fragment at the first meiotic division. At the second division the non-crossover chromatids of such bivalents may be expected to pass to the innermost and outermost nuclei in the egg. The egg nucleus, therefore, receives a single non-crossover strand. Double exchanges presumably do not occur within either inversion.

The importance of the heterozygous inversions in these experiments is owing to their general effect in cutting down crossing over along considerable lengths of the chromosomes, as was first postulated by STURTEVANT (1921). Within *dl-49* only 12 percent chiasmata (that is, 6 percent crossing over) are to be expected on the basis of STURTEVANT and BEADLE'S (1936) studies of attached-X females heterozygous for *dl-49*. No comparable estimate is available for inversion B^{M1}. But inasmuch as B^{M1} is only about ten map units long (see SUTTON 1943), it may confidently be expected that the frequency of crossing over will be at least halved and that at most 10 percent chiasmata will occur in this region. Accordingly not more than 22 percent (as a generous estimate) of all bivalents are expected to be conjoined by chiasmata occurring *within* the inverted regions.

Crossing over to the left of *dl-49* in females heterozygous for *sc v*, *dl-49*, B^{M1} can be estimated from a total of 15,390 flies from various experiments involving both XX and XXY females. Of 8,424 males obtained from *sc v*, *dl-49*, $B^{M1}/y^2\ w^a\ cv\ v\ f$, *Dp112* females by *B*, Swedish-b or Oregon R males, only four were crossovers in this region. Of these only one male carried an exchange in the region between *sc* and w^a. Since 50 percent of such crossovers cannot be detected (that is, y^2 . . . *Dp112* and *sc* . . . *Dp112* are phenotypically indistinguishable), one may assume that perhaps another crossover or so in this region has been missed—although the addition of another exchange to this crossover class will hardly affect the calculated frequency of exchange to the left of *dl-49*. An additional 6,966 flies gave a total of eight males which were crossovers in the region between *sc* and *dl-49*, and in none of these cases were the reciprocal classes unclassifiable. At all events, no more than 13 crossovers to the left of *dl-49* need be accredited in a total of 15,390 flies in which such an exchange could be detected. This gives a crossing over value equal to approximately 0.08 percent. Such a value is far below that of STURTEVANT and BEADLE (1936) for heterozygous *dl-49*. They estimate about 0.5 percent crossing over in this region. Accepting their figure as a possible maximum, no more than 1 percent of all bivalents in the present experiments could have been conjoined by chiasmata to the left of *dl-49*.

Crossing over in the region between the two inversions is almost negligibly rare. Although the map length of this region is approximately 16 units, at most

three crossovers were obtained in a total of 15,390 flies in which such exchanges could be detected. This gives a crossing over frequency of about 0.001 percent. To the right of B^{M1} there were but two crossovers in the same total. In these experiments, therefore, considerably fewer than 1 per cent of the bivalents would be expected to have been conjoined by chiasmata occurring either between the inversions or to the right of B^{M1}. Totalling the exchange percentages for each region—both outside and inside of inversions—no more than 24 percent of all X-chromosome bivalents in *sc v, dl-49,* $B^{M1}/y^2\ w^a\ cv\ v\ f$, *Dp112* females are expected to be held together at metaphase by chiasmata.

According to the chiasma hypothesis of metaphase pairing, the remaining 76 percent of the bivalents which are without chiasmata would fail to remain associated until metaphase or to coorient, and the resulting univalent X's

TABLE 1

Data from matings of sc v, Indl-49, $InB^{M1}/y^2\ w^a\ cv\ v\ f$, *Dp112 by various males; an asterisk (*) indicates that the offspring recorded are derived from egg viability tests.*

MALE PARENT	TOTAL OFFSPRING	TOTAL ♀♀	EXCEP. ♀♀	TOTAL ♂♂	EXCEP. ♂♂	CROSSOVER ♂♂	FREQ. NONDISJUNCT.
ec dx	525	266	1	259	0	0	0.38%
*B**	532	265	0	267	0	0	—
B	2,749	1,424	0	1,325	1	0	0.08%
Swed-b*	1,628	824	3	804	0	0	0.18%
Swed-b	1,694	867	3	827	4	0	0.81%
Ore. R*	3,396	1,798	5	1,598	3	0	0.47%
Ore. R	6,236	3,414	2	2,822	4	4	0.19%
Totals	16,760	8,858	14	7,902	12	4	0.31%
Expected			2,078		1,854		38.0 %

should segregate at random. Thus 50 percent of the egg nuclei resulting from random segregation would be expected to contain one of the X's only, 25 percent would be expected to contain both X's, and the remaining 25 percent should contain neither X chromosome. Fertilizations of the aneuploid eggs will result either in inviable zygotes or in exceptional offspring, depending on the constitutions of the aneuploid egg nucleus and fertilizing sperm (BRIDGES 1916). An egg whose nucleus contains both X chromosomes will give a matroclinous exceptional female when the fertilizing sperm brings in a Y chromosome. Patroclinous male exceptions will occur as sterile XO individuals resulting from fertilizations of the no-X eggs by X-bearing spermatozoa. Most of the XX eggs fertilized by X-bearing spermatozoa will die in the pupal stage, only a few surviving through eclosion and emerging as superfemales. Finally the no-X eggs fertilized by the Y-bearing sperm will die. Since but 24 percent of the X-chromosome synapses of *sc v, dl-49,* $B^{M1}/y^2\ w^a\ cv\ v\ f$, *Dp112* females are expected to give bivalents at first metaphase, there should be produced in

these experiments approximately 11.7 percent matroclinous female exceptions and an equal percentage of patroclinous male exceptions among the adult offspring. Approximately 23.46 percent of the flies of any one sex should be exceptions. The data of table 1 show no agreement with these predictions based upon the chiasma hypothesis of metaphase pairing. There is more than a hundredfold difference between the expectation and the observation. Segregation was essentially normal, there being no significant number of exceptional flies, in spite of the predominance of chiasma-free bivalents.

A supplementary check on these results was carried out to test whether or not there is a high zygotic inviability which could possibly account for the absence from these experiments of the large numbers of adult exceptions expected on the basis of the chiasma hypothesis of metaphase pairing. Table 2 gives the results of these tests of viability, and the crosses marked with asterisks in table 1 record the data on the adults from the same experiments. These findings show that the egg and larval-pupal mortality tests gave the same results as had been obtained from matings in which eggs had not been collected. They *prove* that there is no zygotic inviability which can account for the observed disagreement with the expectations based upon the chiasma hypothesis of metaphase pairing. Crossing over and chiasmata may accordingly be viewed as not necessary requirements for regular disjunction of the X chromosomes of *Drosophila melanogaster*.

TABLE 2

Egg and larval-pupal mortality for inversion heterozygotes; sc v, Indl-49, $InB^{M1}/y^2\ w^a\ cv\ v\ f$, Dp112 by various males.

MALE PARENT	TOTAL EGGS	INVIABLE EGGS	% INVIABLE	HATCHED EGGS	ADULT FLIES	% EMERGENCE OF HATCHED EGGS
B	577	26	4.5	551	532	96.6
Swed-b	1,691	40	2.4	1,651	1,628	98.6
Ore. R	3,573	148	4.1	3,425	3,396	99.1
Totals	5,841	214	3.7	5,627	5,556	98.7
Expected		555	9.5	5,286	4,731	89.5

Comment on the egg-larval-pupal mortality experiments

The first egg collections to be made in these experiments were those of the series in which Oregon R males were used (table 2). Although the egg-inviability of 4.1 percent is by no means an extraordinarily low percentage (STURTEVANT and BEADLE (1936) record 3.1 percent inviable eggs for *dl-49/+×B*, and 1.3 percent inviable eggs for $y^{28}/B\times B$), the 99.1 percent recovery of adults from the hatched eggs awakened immediate suspicion. It seemed possible that some eggs had been overlooked in the initial counts, and thus the percentage emergence of adults recorded for the hatched eggs could be entirely too high. Accordingly, for this and other reasons the *B* and Swedish-b series were run, especial care being taken to avoid a miscount of the eggs. These ex-

periments show essentially the same results as the Oregon R series—namely, low egg mortality and high adult yields from hatched eggs (table 2). There may still be a small undetected error, but the confidence limits for P=0.99 indicate that for these experiments about 95 percent of all of the eggs from the inversion heterozygotes hatch, and that over 97 percent of the hatched eggs develop into adults.

DISCUSSION

The improbability of a chiasma interpretation of segregation in inversion heterozygotes

The following facts derivable from the experiments described above seem indisputable. (1) The great preponderance of X-chromosome tetrads formed by synapsis of *Indl-49*, InB^{M1} and *Dp112* chromosomes are not associated by simple exchanges or by chiasmata in either the long left limb (X^L) or in the minute right limb (X^R). (2) In spite of the high frequency of non-exchange tetrads ($\geqq 76$ percent), or bivalents not conjoined by chiasmata, segregation of the X chromosomes is essentially normal in the female. These facts are jointly opposed to the chiasma hypothesis of metaphase pairing. STURTEVANT and BEADLE (1936), GERSHENSON (1935), and STONE and THOMAS (1935) from similar experimental results have concluded that exchanges (hence chiasmata) are not necessary for normal disjunction of the X chromosomes in female *Drosophila melanogaster*. Probably but one serious criticism of the collective results from the study of heterozygous inversions in female Drosophila, and the conclusions drawn therefrom, is likely to be voiced in defense of the chiasma hypothesis of metaphase pairing. It may be contended by some that the designs of these experiments do not eliminate the possibility that undetected reciprocal exchanges in the so-called inert regions proximal to the kinetochores may in fact have been the causal agents of segregation in otherwise apparently non-crossover bivalents.[2]

[2] MATHER (1944) has recently published data which he contends require for their interpretation the regular occurrence of reciprocal chiasmata between X and Y in the male. For the following reasons MATHER neither establishes support for the reciprocal chiasmata hypothesis, nor demonstrates a "polygenic" constitution for the Y chromosomes studied.

(1) The genetic technique employed by MATHER is outmoded and gives uncertain isogenicity at the fifth generation with which he initiates his experiments. The expression $(1-2^{-n})^3$ for calculating the chance of isogenicity for all three autosomes after n generations of backcrossing is strictly applicable only if a single male is used per culture. MATHER's account makes clear only that at least two males per culture were used at the fifth and succeeding backcrosses. A ***minimum*** of ten males, therefore, were selected from the fourth generation for backcrossing of the experimental Y-chromosome stocks, producing the fifth or initially measured generation. If single males were employed before the fifth backcross, then the probability (P) that at least three males among the parents of the fifth generation carry one or more unreplaced autosomes is:

$$1-[p^{10}+10p^3(1-p)+45p^8(1-p)^2],$$

where $p=(1-2^{-4})^3=0.82$. Hence P=0.25, or the chance is 1 in 4 that at least two Y-chromosome strains may be expected to produce sons having one or more unreplaced autosomes at the fifth generation.

On the other hand, if in fact two males per culture were employed in every backcross, and if

Both GERSHENSON and STURTEVANT and BEADLE considered the remote possibility of explaining their results by such undetectable exchanges, and rejected such an interpretation as highly improbable. GERSHENSON's analysis consisted of showing that such reciprocal exchanges could be held accountable for normal segregation only by ignoring the data then available for crossing over to the right of bobbed, and that synapsis of *InClB*/+ chromosomes probably for the most part interferes with the pairing of the proximal regions. STURTEVANT and BEADLE, however, supplied three sets of data which collectively seem more than sufficient to exclude the possibility of reciprocal exchanges between the proximal inert regions. *InDf(bb)* carries an inverted segment extending from between *rb* and *rg* to between *car* and the kinetochore (STURTEVANT and BEADLE 1936). This chromosome furthermore possesses a deficiency for the proximal third of the somatic X chromosome, including the locus of bobbed (SIVERTZEV-DOBZHANSKY and DOBZHANSKY 1933). In spite of the loss of most of the region in which the supposed reciprocal exchanges are to occur, in spite of an upset of homology in the remaining fraction of the inert region by the included inversion, and in spite of the inevitable occurrence of non-exchange tetrads owing to the inversion, *InDf(bb)*/+mothers gave no matroclinous daughters in a total of 1,244 female offspring. In this instance it would be nothing short of fantasy to maintain that reciprocal exchanges in the deleted and partially inverted proximal region underlie normal segregation of the X chromosomes.

Less striking but additional supporting evidence that reciprocal exchanges are not involved follows from STURTEVANT and BEADLE's studies of the *sc-8* inversions. *Insc-8* and *InDf(sc-8)* likewise upset homology well within the inert region to the right of *bb*. Such an upset, from what is known concerning crossing over in regions immediately adjacent to inversions, must necessarily

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isogenic and heterogenic males breed alike, then the chance (p_n) that both male parents of a given strain are isogenic for all three autosomes at generation n is:

$$\left[1-\frac{5\times 4^n-2}{3\times 8^n}\right]^3$$

From this it follows that the chance that any one strain (such as, Y^{Ok}) is isogenic at the fifth backcross is 0.72, and the chance that two or more strains have one or more unreplaced autosomes is 2 in 5. In either case there is a very great likelihood that MATHER's Y-chromosome stocks were not isogenic at the start of his experiments.

(2) There is an evident lack of adequate environmental control.

(3) MATHER (page 320, line 4) states that there is no "external evidence" that unreplaced autosomes produce different effects in the two sexes. It should also be noted that there is likewise no external evidence for bristle modifiers in the Y chromosomes of his stocks or for reciprocal exchanges between X and Y.

(4) Internal contradictions, such as the failure of Y^{Or} to change $\widehat{XX}$ chromosomes in 22 generations (expt. 4) of association, exist within experiments.

(5) Theoretical considerations (even ignoring the weighty objections to constant reciprocal exchanges), such as the fact that both arms of Y pair with X,—and presumably with different frequencies (NEUHAUS 1936, 1937),—likewise shed grave doubts on the validity of MATHER's conclusions.

interfere with, and markedly lower, crossing over in the proximal segment. If reciprocal chiasmata are supposed to occur in this proximal segment, it must also be recognized that the *sc-8* inversions will have a suppressive action upon them. Accordingly, some percentage of the bivalents which would be expected to be conjoined solely by reciprocal chiasmata must in fact have no chiasmata occurring between the homologs. These, on the chiasma hypothesis, must fail to coorient and thus assort at random, with the result that 25 percent of their segregations terminate in the production of eggs which will give rise to matroclinous daughters when fertilized by Y-bearing spermatozoa. Experimentally it is found, however, that *Insc-8*/+ and *InDf*(*sc-8*)/+ give no higher frequency of exceptional daughters than does a normal female (0.02 percent for *Insc-8*/+; 0.31 percent for *InDf*(*sc-8*)/+; total, more than 5,000 female offspring). The same general disagreement with a chiasma interpretation of segregation was obtained in the present study in which InB^{M1} likewise upsets homology within the inert region (SUTTON 1943). In spite of this, no more than 0.16 percent exceptional daughters actually occurred among the 8,858 female offspring.

There seems to be but one conclusion to be drawn which is in harmony with the experimental results—namely, chiasmata are *not* a necessary condition for segregation in female Drosophila, although when present in a bivalent they may be a sufficient cause of segregation. Whatever the normal or average architecture of a bivalent may be in female Drosophila homozygous for normal or inverted chromosomes, it seems certain that the preponderance of bivalents in *Indl-49*, $InB^{M1}/Dp112$ and other inversion heterozygotes may be without chiasmata and nevertheless undergo normal disjunction.

The modes of conjunction at meiosis

Any mechanism which makes possible almost invariable separation of homologous kinetochores (hence chromosomes) to alternative cells at meiosis will guarantee segregation. There can be little doubt that crossing over with resulting chiasma formation supplies a means for doing just this. But neither can there be any doubt that the primary mechanism which brings about synapsis itself may likewise provide a device ensuring segregation if the paired condition is retained until metaphase. Very possibly the paired synaptic state can persist to metaphase in organisms having very small or little-coiled chromosomes at meiosis, whether or not chiasmata are formed. The principal significance of crossing over is that it provides both a mechanism giving recombination of genes as well as a means for preventing too great genetic divergence of initially identical chromosomes.

The assurance of segregation by mechanical ties through persistent chiasmata is almost certainly a secondary attribute. If, in evolution, increasing complexity in structure, or physiology, or even increasing bulk of a chromosome were to interfere with an established mode of conjunction derived from the synaptic mechanism, then there would be positive selection for any sec-

ondary mechanism or mechanisms which would serve to make the new karyotype stable in its meiotic behavior. Thus persistence of chiasmata, increase in shared matrix or pellicle, development of adhesive mechanisms (as telomeres and collochores may prove to be), or exploitation of what appears to be pairing by heteropycnotic structures—all such devices could serve in achieving persistent regularity of coorientation and segregation in spite of increasing divergence of the chromosome from its ancestral prototype and behavior. At first glance the chiasma appears to be the most likely mechanism to be fostered by such selection owing to the general ubiquity of crossing over at meiosis. Yet where is found the metaphase chromosome which is devoid of matrix and other components presumably accessory to the genonema and which by modification may serve as segregative mechanisms at meiosis? Since there are organisms in which crossing over is suppressed in one sex, it is clear that chiasmata are not alone in possessing the virtue of being capable of ensuring regularity of segregation. It is equally clear that selection has preserved devices guaranteeing segregation without the mediation of chiasmata, as has been here proven for the Drosophila female, even in organisms where crossing over is the rule. There seems little reason, therefore, to ignore the mechanisms of conjunction which assuredly replace or supplement chaismata in mechanically ensuring segregation in many organisms. To reason to a universal chiasma hypothesis has proven as unsound as the nineteenth century attempt to rigidly define a substance "protoplasm." It is no surprise that the chiasma proves only to be a sufficient—not a necessary—cause for segregation in some organisms, while in others, such as the lilies, of all possible mechanisms only chiasmata appear to be capable of guaranteeing segregation. What is surprising is the fact that alternative mechanisms which have been so carefully studied and described by the morphological school of cytologists have been so unwisely ignored or even discredited by many geneticists and cytogeneticists without further investigation.

SUMMARY

Approximately 76 percent of the bivalents formed by *Indl-49*, InB^{M1}/Dp_{112} X-chromosome heterozygotes are not conjoined by chiasmata.

In spite of the high frequency of non-exchange tetrads, or bivalents not conjoined by chiasmata, segregation of the X chromosomes is essentially normal in the female.

The chiasma hypothesis of metaphase pairing is not universally applicable, even if the domain generalized by it is specified in such a manner that aberrant forms of meiosis are deliberately excluded.

Mechanisms, other than chiasmata, which may serve to guarantee meiotic conjunction and segregation are briefly discussed.

Recent experimental work interpreted on the basis of the reciprocal chiasmata hypothesis of X-Y conjunction at meiosis in Drosophila males is shown neither to require nor to support that hypothesis.

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LITERATURE CITED

BAUER, H., 1939 Chromosomenforschung (Karyologie und Cytogenetik). Fortschr. Zool., N.F. **4**: 584–597.

BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1**: 1–52, 107–163.

COOPER, K. W., 1938 Concerning the origin of the polytene chromosomes of Diptera. Proc. Nat. Ac. Sci. **24**: 452–458.

1944 Analysis of meiotic pairing in Olfersia and consideration of the reciprocal chiasmata hypothesis of sex chromosome conjunction in male Drosophila. Genetics **29**: 537–568.

DARLINGTON, C. D., 1929 Meiosis in polyploids. II. Aneuploid hyacinths. J. Genet. **21**: 17–56.

1930 A cytological demonstration of "genetic" crossing over. Proc. Roy. Soc. London B **107**: 50–59.

1931a Meiosis. Biol. Rev. **6**: 221–264.

1931b Meiosis in diploid and tetraploid *Primula sinensis*. J. Genet. **24**: 65–96.

1932 Recent advances in cytology. 559+18 pp. Philadelphia: Blakiston.

1939 The evolution of genetic systems. 149+11 pp. Cambridge: Cambridge University Press.

DOBZHANSKY, TH., 1932 Cytological map of the X-chromosome of *Drosophila melanogaster*. Biol. Zbl. **52**: 493–509.

GERSHENSON, S., 1935 The mechanism of non-disjunction in the *ClB* stock of *Drosophila melanogaster*. J. Genet. **30**: 115–125.

HUGHES-SCHRADER, S., 1940 The meiotic chromosomes of the male *Llaveiella taenechina* Morrison (Coccidae) and the question of the tertiary split. Biol. Bull. **78**: 312–337.

1943a Meiosis without chiasmata—in diploid and tetraploid spermatocytes of the mantid *Callimantis antillarum* Saussure. J. Morph. **73**: 111–141.

1943b Polarization, kinetochore movements, and bivalent structure in the meiosis of male mantids. Biol. Bull. **85**: 265–300.

HUSKINS, C. L., 1937 The internal structure of chromosomes. A statement of opinion. Cytologia. Fujii Jubilee Volume: 1015–1022.

JANSSENS, F. A., 1909 Spermatogénèse dans les batraciens. V. La théorie de la chiasmatypie, nouvelle interpretation des cinèses de maturation. Cellule **25**: 387–411.

1919a A propos de la chiasmatypie et la théorie de Morgan. C. R. Soc. Biol. Paris. **82**: 917–920.

1919b Une formule simple exprimant ce qui se passe en réalité lors de la "chiasmatypie" dans les deux cinèses de maturation. C. R. Soc. Biol. Paris **82**: 930–934.

1924 La chiasmatypie dans les insectes. Spermatogénèse dans 1° *Stethophyma grossum* (L), 2° *Chorthippus parallelus* (Zetterstedt). Cellule **34**: 135–359.

KAUFMANN, B. P., 1934 Somatic mitoses of *Drosophila melanogaster*. J. Morph. **56**: 125–155.

MATHER, K., 1944 The genetical activity of heterochromatin. Proc. Roy. Soc. London B **132**: 308–332.

NEUHAUS, M., 1936 Crossing-over between the X and Y-chromosomes in the female of *Drosophila melanogaster*. Z.i.A.V. **71**: 265–275.

1937 Additional data on crossing over between X and Y chromosomes in *Drosophila melanogaster*. Genetics **22**: 333–339.

PROKOFIEWA, A., 1935 Morphologische Struktur der chromosomen von *Drosophila melanogaster*. Z. Zellf. mikr. Anat. **22**: 255–262.

SAX, K., 1936 Chromosome coiling in relation to meiosis and crossing over. Genetics **21**: 324–338

SCHRADER, F., 1944 Mitosis. The movements of chromosomes in cell division. 110+10 pp. New York: Columbia University Press.

SIVERTZEV-DOBZHANSKY, N. P., and TH. DOBZHANSKY, 1933 Deficiency and duplications for the gene bobbed in *Drosophila melanogaster*. Genetics **18**: 173–192.

SPASSKY, B., 1943 Cream of wheat-molasses fly medium. Drosophila Information Service **17**: 67–68.

STONE, W., and I. THOMAS, 1935 Crossover and disjunctional properties of X chromosome inversions in *Drosophila melanogaster*. Genetica **17**: 170–184.

STURTEVANT, A. H., 1921 A case of rearrangement of genes in *Drosophila*. Proc. Nat. Acad. Sci. **7**: 235–237.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

SUTTON, E., 1943 Bar eye in *Drosophila melanogaster:* A cytological analysis of some mutations and reverse mutations. Genetics **28**: 97–107.

WILSON, E. B., and T. H. MORGAN, 1920 Chiasmatype and crossing over. Amer. Nat. **54**: 193–219.

SUMMARIES FROM FORTHCOMING PAPERS

IRWIN, M. R., and L. J. COLE. *Evidence for normal segregation of species-specific antigens in the backcross of species hybrids in doves.*—Received May 17, 1945.—The antigens specific to Senegal, as contrasted to Ring dove, appeared to segregate at random in the first backcross generation; at least the segregation was not sufficiently at variance with expectation according to chance alone to be detected. Because the distribution of these cellular characters of Senegal appeared to be normal, it may be concluded that there was more or less normal assortment in the species hybrid parents of the nine or more chromosomes of Senegal carrying genes for antigens specific to the species. This in turn suggests that there was more or less normal pairing of these nine chromosomes of Senegal with partners in Ring dove.

MAMPELL, KLAUS. *Analysis of a mutator.*—Received May 18, 1945.—A recessive mutator gene in *Drosophila pseudoobscura* is the object of the investigation. Minutes and Minute mosaics are characteristic mutations in the cultures showing the high mutability. The mutator is in chromosome II, in the vicinity of cinnabar. The mosaics represent somatic mutations; when those mutations affect the germ line, they are inherited as single genes. On outcrossing, only males produce mosaics in their offspring. Possible reasons for the failure of the females to produce the mutations are presented. It is assumed that the mutator when homozygous produces a cytoplasmic component which induces the mutation. The component is transmitted through the cytoplasm of the sperm. This represents a "paternal effect." The mutation rates resulting from the various possible crosses are discussed. Differential susceptibility of the genes to the mutator may account for the different levels of mutability. "Mutation fashions" can be explained on the basis of this susceptibility. It is suggested that the carrier of mutability is evenly distributed through the cytoplasm.

GREGORY, P. W., W. M. REGAN, and S. W. MEAN. *Evidence of genes for female sterility in dairy cows.*—Received May 23, 1945.—A qualitative analysis of female fertility and sterility was made for all Jerseys and Holsteins of the California Experiment Station herd that have reached the age of reproduction during the past 25 years. The Jerseys involve 266 cows sired by 16 bulls; the Holsteins, 94 cows sired by 2 bulls. The terms fertility and sterility as used in this report are defined. All the sterility observed was limited to females. No quantitative study of female fertility was attempted.

Only a few of the bulls produced sufficient progeny for an adequate genetic analysis. The use of sire-daughter matings permitted a Mendelian analysis for genes conditioning female sterility.

Five Jersey bulls and one Holstein produced a total of 20 sterile daughters from outcrossed and inbred matings. Three of the original, unrelated Jersey

sires had sterile daughters. The available data, however, permitted an analysis of only two; one of the original sires was analyzed directly, and the other indirectly by means of a son and a grandson. The Holstein bull that produced sterile heifers was used extensively in sire-daughter matings. The female sterility transmitted by each of the bulls tested followed a single factor recessive autosomal sex-limited pattern of inheritance.

It was not determined whether the original bulls carried the same or different genes for female sterility. Judging from the evidence, the original population of Jersey and Holstein cows carried a gene for female sterility, the frequency value being approximately .1 or less.

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